UNIVERSITY OF ALBERTA

Synthesis of Galactofuranosides for the Characterization of Galactofuranosyltransferases Involved in Mycobacterial Arabinogalactan Biosynthesis

by

Gladys Cherisse J. Completo

A thesis submitted to the Faculty of Graduate Studies and Research

in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

DEPARTMENT OF CHEMISTRY

Edmonton, Alberta

Fall, 2008

Library and Archives Canada

Published Heritage Branch

395 Wellington Street
Ottawa ON K1A 0N4
Canada

Bibliothèque et Archives Canada

Direction du Patrimoine de l'édition

395, rue Wellington
Ottawa ON K1A 0N4
Canada

Your file Votre référence
ISBN: 978-0-494-46300-0
Our file Notre référence
ISBN: 978-0-494-46300-0

NOTICE:
The author has granted a non-exclusive license allowing Library and Archives Canada to reproduce, publish, archive, preserve, conserve, communicate to the public by telecommunication or on the Internet, loan, distribute and sell theses worldwide, for commercial or non-commercial purposes, in microform, paper, electronic and/or any other formats.

The author retains copyright ownership and moral rights in this thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without the author's permission.

AVIS:
L'auteur a accordé une licence non exclusive permettant à la Bibliothèque et Archives Canada de reproduire, publier, archiver, sauvegarder, conserver, transmettre au public par télécommunication ou par l'Internet, prêter, distribuer et vendre des thèses partout dans le monde, à des fins commerciales ou autres, sur support microforme, papier, électronique et/ou autres formats.

L'auteur conserve la propriété du droit d'auteur et des droits moraux qui protège cette thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

In compliance with the Canadian Privacy Act some supporting forms may have been removed from this thesis.

While these forms may be included in the document page count, their removal does not represent any loss of content from the thesis.

Conformément à la loi canadienne sur la protection de la vie privée, quelques formulaires secondaires ont été enlevés de cette thèse.

Bien que ces formulaires aient inclus dans la pagination, il n'y aura aucun contenu manquant.

Canada

To Ruel and Isabelle... and most especially to my late dad, Cesar

Abstract

Arabinogalactan (AG) is a unique complex comprised of D-galactofuranosyl and D-arabinofuranosyl monosaccharides that is present in the mycolyl-arabinogalactan complex (mAG) of the *Mycobacterium tuberculosis* cell wall. The AG complex is essential for mycobacterial viability and, thus, the enzymes involved in its biosynthesis might serve as putative therapeutic targets. The galactan chain is composed of ~30 D-galactofuranose (Gal*f*) residues that are linked via alternating β-(1→5) and β-(1→6) linkages with the reducing end covalently attached to a linker disaccharide consisting of rhamnose and N-acetylglucosamine. A series of oligosaccharides (**1–12**) were synthesized to probe the specificity of two galactosylfuranosyltransferases, GlfT1 and GlfT2, involved in the biosynthesis of the cell wall galactan chain. The synthesis of the galactofuranosyl monosaccharide moiety was achieved via an iodine-promoted cyclization of galactose diethyl dithioacetal using an alcohol solvent and free of the usual pyranoside side product. The general synthetic approach used to prepare most of these compounds involved coupling reactions between benzoyl-protected thioglycoside donors and octyl glycoside acceptors. The preparation of trisaccharides and tetrasaccharides **5–8**, however, utilized synthetic routes wherein these compounds were assembled starting from the reducing to nonreducing end and vice versa. The need to produce compounds **5** and **6** in large scale, for use in biochemical assay experiments, necessitated the development of a one-pot method for their synthesis.

Compounds **1–8** were also used to probe the specificity of GlfT2, originally known as glfT, a bifunctional galactofuranosyltransferase catalyzing the formation of both β-(1→5) and β-(1→6) Gal*f* linkages in mycobacterial galactan. Trisaccharide **5** (β-Gal*f*-(1→5)-β-Gal*f*-(1→6)-β-Gal*f*-Octyl) was determined as the best substrate of the GlfT2-catalyzed reaction. Likewise, the first ever synthesis of four mAG fragments (**9–12**), made up of the linker disaccharide decorated with one, two or three Gal*f* residues, was accomplished through coupling of a protected linker disaccharide derivative (**112**) with a mono-, di-, or tri-galactofuranosyl thioglycoside (**43**, **106**, or **99**, respectively). These compounds (**9–12**) were used to probe a newly identified galactofuranosyltransferase, GlfT1, known to have dual β-(1→4) and β-(1→5) Gal*f* transferase activities during the initial steps of galactan formation. The results showed that only two bifunctional galactofuranosyltransferases GlfT1 and GlfT2, are involved in the assembly of mycobacterial galactan.

ACKNOWLEDGMENT

The making of this dissertation is a joint effort of so many people who deserve my heartfelt appreciation and gratitude.

First and foremost, I would like to thank my advisor, Dr. Todd Lowary, without his constant guidance and provision; I could not have finished this dissertation. I feel fortunate to have had the chance to work with him and learn from him. Over the years, our relationship has gone beyond typical student-advisor relationship. I value his friendship, at the same time for pushing my capabilities of becoming a better researcher and I will always bear in mind all that I have learnt from him. My appreciation for his continuous support and guidance about my future career is immeasurable.

I would like to thank Dr. David Bundle and Dr. Frederick West for their support and guidance not just by serving in my committee but for providing assistance while I was looking for a postdoctoral position. I will remain in awe for their intellect and dedication to science.

I would like to thank Dr. Robert Campbell, Dr. John Klassen, Dr. Mark Peppler and Dr. Jacquelyn Gervay-Haque for serving in my committee and for their helpful suggestions.

I would like thank the former and current group members, Jon, Han, Prakriti, Maju, Yu Bai, Wei Shi, Hyosun, Eric, Jayant, Dianjie, Chunjuan, Jing, Tony, Ruokon, Hashem, Yanqing, Tish, Blake, Jean, Jessica, Daniel, Jared, Nick, Mickey and Simon for their help

and company and for making coming to the lab more exciting and enjoyable.

I appreciate the help from the staff of the chemistry department, especially, Lynne Lechelt, Dr. Albin Otter, Mark, Dr. Randy Whittal, Angie, Wayne, Jeanette, Ilona, and Karen.

I would like to thank my family, my beloved parents Gwen and Cesar, sister Gayle, and brothers Cyril and Cyrus for their unwavering support and for always believing in my goals and dreams.

I would like to take this opportunity to express my profound gratitude to Ruel, my husband, friend and mentor, for the unconditional support, for being always there for me through thick and thin.

To Isabelle, my inspiration- this is for you my precious one.

TABLE OF CONTENTS

Chapters Page

1. Introduction.....1

Tuberculosis.....2

Drugs Used to Treat Tuberculosis.....3

Pathogenesis of Tuberculosis.....5

Structure of the Mycobacterial Cell Wall.....9

1.4.1 Peptidoglycan.....10

1.4.2 Mycolyl-Arabinogalactan-Peptidoglycan (mAGP) Complex.....11

1.4.2.1 Arabinogalactan (AG).....12

1.4.2.2 Mycolic Acids.....14

1.4.3 Lipoarabinomannan (LAM) and Lipomannan (LM).....16

1.4.4 Extractable Lipids.....18

1.5 Biosynthesis of the Mycolyl-Arabinogalactan-Peptidoglycan Complex.....19

1.6 Cell Wall Core Galactan as a Potential Target for the Development of New Anti-TB Drugs.....22

1.6.1 UDP-Galactofuranosyltransferase 2 (GlfT2).....23

1.6.2 UDP-Galactofuranosyltransferase 1 (GlfT1).....28

1.7 Project Goals.....31

2. Results and Discussion.....32

2.1 Introduction – Methods for Accessing Galactofuranose Derivatives.....33

2.1.1 Fischer Glycosylation.....33

2.1.2 High Temperature Acylation.....35

2.1.3 Regioselective Opening of 1,4-Anhydrogalactopyranose Derivatives.....35

2.1.4 Synthesis of Di-*O*-isopropylidene Derivatives.....36

2.1.5 Cyclization of Dithioacetals.....38

2.2 Synthetic Strategy.....40

2.3 Preparation of Thioglycoside Donors.....43

2.4 Preparation of Octyl Glycoside Acceptors.....54

2.5 Synthesis of Octyl Galactofuranosides Acceptors.....55

2.6 Synthesis of Oligosaccharides.....61

2.6.1 Synthesis of Disaccharides **1–4**.....61

2.6.2 Synthesis of Trisaccharides **5** and **6**.....65

2.6.3 Synthesis of Tetrasaccharides **7** and **8**.....70

2.7 New Approach for the Synthesis of Tetrasaccharides **7** and **8**.....78

2.7.1 Synthesis of Trichloroacetimidate **96**.....79

2.7.2 Synthesis of Disaccharides **97** and **106**.....81

2.7.3 Synthesis of Trisaccharides **99** and **110**.....82

2.7.4 Synthesis of Tetrasaccharides **7** and **8**.....85

2.7.5 One-pot Syntheses of Trisaccharides **5** and **6**.....87

2.7.6 Retrosynthetic Analysis for One-pot Syntheses of Trisaccharides **5** and **6**.....88

2.7.7 One-pot Syntheses of Trisaccharides **5** and **6**.....90

2.8 Characterization of GlfT2 using Oligosaccharides **1–8**.....93

2.8.1 Kinetic Characterization of **1–6**.....94

2.8.2 Characterization of Products obtained from **5** and **6**.....97

2.9 Formulation of a Hypothesis for Galactan Biosynthesis 100

2.10 Synthesis of Oligosaccharides with Linker Disaccharide 103

2.10.1 Retrosynthetic Analysis for **9–12**.....103

2.10.2 Synthesis of Disaccharide **9**.....105

2.10.3 Synthesis of Oligosaccharides **10–12**.....107

2.11 Probing the Role of GlfT1 and GlfT2 in Galactan Biosynthesis with **9–12**.....111

2.12 Conclusions.....117

2.13 Future Work.....119

3. Experimental Procedures.....128

Bibliography.....215

LIST OF TABLES

Table		Page
1	Synthesis of **35** as a function of iodine concentration	47
2	Acceptor specificity of recombinant GlfT2 using **1–6**	95
3	Ability of **9–12** to serve as acceptors for GlfT2	112

LIST OF FIGURES

Figure		Page
1	Structures of selected front-line anti-TB drugs in clinical use	4
2	Pathogenesis of tuberculosis	6
3	TB Drugs targeting distinct *M. tuberculosis* subpopulations	8
4	Schematic depiction of the mycobacterial cell wall	10
5	*N*-acetylglucosamine and *N*-glycolylmuramic acid linkages in the peptidoglycan	11
6	Schematic drawing of the structure of mycolyl-AG complex	12
7	Structural motifs in mycobacterial mycolyl-AG complex	13
8	Representative structures of mycolic acids	16
9	Schematic representation of mycobacterial lipoarabinomannan (LAM)	17
10	Representative examples of extractable lipids	19
11	Biosynthetic pathway for mAGP complex	21
12	Representative reaction catalyzed by GlfT2.	24
13	Proposed mechanism of inverting GlfT2	25
14	Reaction catalyzed by UDP-Gal*p* mutase	26
15	Synthesized compounds used to probe specificity of GlfT2	28
16	Galactofuranosyl linkages present in the galactan	29
17	Compounds synthesized containing the linker unit	30

18 Pseudorotational itinerary for a D-galactofuranose ring.....50

19 Conformations of 1-*O* and 1-*S*-galactofuranosides based on $J_{1,2}$.....51

20 Radiochemical assay for GlfT2.....94

21 Oligosaccharides **1–6** screened as substrates for GlfT2.....95

22 MALDI mass spectra of large-scale incubation mixtures of **5** (A) or **6** (B) with UDP-Gal*f* and GlfT2 97

23 Partial ^{1}H NMR spectra of tetrasaccharides produced from large-scale incubation of **5** (A) or **6** (B) with UDP-Gal*f* and GlfT2.....99

24 Structure of mycobacterial galactan attached to the linker disaccharide.....101

25 Proposed model for galactan assembly.....102

26 Galactan biosynthetic precursors isolated from mycobacteria.....113

27 Results of incubating **9–12** with UDP-Gal*f* in the presence of GlfT1.....114

28 Steps involved in the biosynthesis of mycobacterial galactan.....115

29 Potential substrates and/or inhibitors of GlfT1.....119

30 Potential inhibitors of GlfT2.....121

31 Proposed one-pot synthesis of potential inhibitor of GlfT2.....122

32 Potential inhibitors of GlfT2 with conformationally restricted core structures.....123

33 Minimum energy-optimized structure of galactofuranose ring containing 2,3,anhydrosugar residue.....123

34 Proposed donor analogs of UDP-Gal*f*.....124

35 Potential phosphate analogs.....125

36 Potential substrates to probe binding of the acceptor with GlfT2.....126

LIST OF SCHEMES

Scheme		Page
1	Fischer glycosidation of D-galactose	34
2	Modified synthesis of *n*-pentenyl galactofuranosides	34
3	High temperature benzoylation of D-galactose	35
4	Ring opening of 1,4-anhydro-2,3,6-tri-*O*-benzoyl-β-D-galactose	36
5	Synthesis of 1,2:5,6-di-*O*-isopropylidene-α-D-galactofuranose **24**	36
6	Cyclization of *S,O* acetals to give ethyl β-D-galactofuranoside	37
7	Synthesis of galactofuranosides via cyclization of open-chain *S,O*-acetals	38
8	DBDH-mediated dithioacetal cyclization with methanol	39
9	Treatment of D-glucose diethyl dithioacetal with iodine and methanol	39
10	Monosaccharide building blocks	41
11	Preparation of methyl D-galactofuranoside **35** via Fischer glycosylation	43
12	Attempted high temperature benzoylation of D-galactose	44
13	Galactofuranose derivatives via reduction of benzoylated galactonolactone	44
14	Proposed mechanism of iodine-induced dithioacetal cyclization in methanol	46
15	Preparation of thioglycoside **57** via cyclization of dithioacetal **34**	47
16	Anchimeric assistance of a benzoyl group leading to 1,2-*trans*-selectivity	49
17	Synthesis of donors **41** and **42**	52
18	Acyl migration of benzoyl group from O-5 to O-6 under acidic conditions	53

19 Synthesis of thioglycoside donor **44**.....54

20 Structures of octyl glycoside acceptors **39** and **40**.....55

21 Synthesis of octyl glycoside **70** from donor **43**.....56

22 Synthesis of octyl glycoside via dithioacetal cyclization.....57

23 Isopropylidene protection of octyl glycoside **72**.....58

24 Synthesis of octyl glycosides **39** and **40**.....59

25 Preparation of octyl glycosides **39** and **40** using TFA.....60

26 Synthesis of disaccharides **1** and **2**.....62

27 Synthesis of disaccharides **3** and **4**.....64

28 Retrosynthetic analysis for the preparation of trisaccharides **5** and **6**.....66

29 Synthesis of trisaccharide **5**.....67

30 Synthesis of trisaccharide **6**.....69

31 Synthesis of tetrasaccharide **7**.....71

32 Attempted synthesis of tetrasaccharide **8** via 1 + 3 coupling strategy.....73

33 Attempted synthesis of tetrasaccharide **95** via 2 + 2 thioglycoside coupling 75

34 Attempted synthesis of tetrasaccharide **95** via 2 + 2 imidate coupling.....76

35 Attempted synthesis of tetrasaccharide **95** via 3 + 1 imidate coupling.....77

36 Retrosynthetic analysis of tetrasaccharide **8** via alternate strategy 78

37 Synthesis of galactofuranosyl disaccharides using trichloroacetimidate **100**.....80

38 Synthesis of trichloroacetimidate **96**.....81

39 Synthesis of disaccharide **97** using trichloroacetimidate **96**.....81

40 Synthesis of disaccharide **106** using trichloroacetimidate **96**.....82

41 Synthesis of trisaccharide **99**.....83

42 Synthesis of trisaccharide **110**.....84

43 Synthesis of tetrasaccharide **8**.....85

44 Synthesis of tetrasaccharide **7**.....86

45 Retrosynthetic analysis for one-pot syntheses of trisaccharides **5** and **6**.....89

46 One-pot synthesis of trisaccharide **5**.....91

47 One-pot synthesis trisaccharide **6**.....92

48 Oligosaccharide targets **9–12**, which contain the α-L-Rha*p*-(1→3)-α-D-Glc*p*NAc linker disaccharide 102

49 Retrosynthetic analysis for **9–12**.....104

50 Synthesis of disaccharide **9**.....106

51 Synthesis of acceptor **112**.....107

52 Synthesis of Trisaccharide **10**.....109

53 Synthesis of tetrasaccharide **11**.....110

54 Synthesis of pentasaccharide **12**.....111

LIST OF ABBREVIATIONS

Abbreviation	Full Name of Compound
Ac	Acetyl
AcCl	Acetyl chloride
Ac_2O	Acetic anhydride
AcOH	Acetic acid
aq.	Aqueous
Ara*f*	arabinofuranosyl
AG	Arabinogalactan
Bn	Benzyl
br s	broad singlet
BSP	1-benzenesulfinyl piperidine
Bu	Butyl
Bz	Benzoyl
BzCl	Benzoyl chloride
° C	Degree Celsius
cat.	Catalytic
COD	Cyclooctadiene
CSA	Camphorsulfonic acid

D	doublet
Dd	doublet of doublets
ddd	doublet of doublet of doublets
DBDH	Dibromo-5,5-dimethylhydantoin
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DIBAL-H	Diisobutylaluminum hydride
DMAP	4-Dimethylaminopyridine
DMDO	Dimethyldioxirane
DMF	*N*,*N*'-Dimethylformamide
DMSO	Dimethyl sulfoxide
DMTST	Dimethyl(methylthio)sulfonium triflate
dt	doublet of triplets
DTBMP	2,6-di-*tert*-butyl-4-methylpyridine
EDC	*N*-(3-Dimethylaminopropyl)-*N*'-ethylcarbodiimide
Et	Ethyl
EtOH	Ethanol
Gal*f*	Galactofuranosyl
GlfT1	UDP-Galactofuranosyltransferase I
GlfT2	UDP-Galactofuranosyltransferase II
HIV-AIDS	Human Immunodeficiency Virus-Acquired

	Immunodeficiency Syndrome
HMBC	Heteronuclear Multiple Bond Correlation Experiment
Hz	Hertz
IBX	2-Iodoxybenzoic acid
IDCP	iodonium dicollidine perchlorate
LAM	lipoarabinomannan
LM	lipomannan
m	multiplet
m-CPBA	*meta*-Chloroperoxybenzoic acid
mAG	mycolyl-arabinogalactan (mAG)
mAGP	mycolyl-arabinogalactan-peptidoglycan complex
MDR-TB	Multi drug resistant TB
Me	Methyl
Ms	Methanesulfonyl
MTB	Mycobacterium tuberculosis
NBS	*N*-bromosuccinimide
NMR	Nuclear magnetic resonance
NIS	*N*-iodosuccinimide
Ph	Phenyl

PMB	4-Methoxybenzyl
ppm	Parts per million
p-TsOH	Para-toluenesulfonic acid
q	quartet
s	singlet
Salen	Salisaldehyde ethylenediamine
t	triplet
TB	tuberculosis
TBAF	Tetrabutylammonium fluoride
TBDMS	*tert*-Butyldimethylsilyl
TBDPS	*tert*-Butyldiphenylsilyl
TESOTf	*tri*-ethylsilyltrifluoromethanesulfonate
Tf	Trifluoromethanesulfonyl
TFA	Trifluoroacetic acid
TfOH	Trifluoromethanesulfonic acid
Tf_2O	Trifluoromethanesulfonic anhydride
THF	Tetrahydrofuran
TMS	Trimethylsilyl
TMSOTf	*tri*-methylsilyltrifluoromethanesulfonate
Tol	Tolyl

TolSH	4-methylbenzenethiol
TPAP	Tetrapropyl ammonium perruthenate
Tr	Triphenylmethyl;trityl
UDP	Uridine diphosphate
XDR-TB	extensively drug resistant TB

CHAPTER 1

INTRODUCTION

1.1 Tuberculosis

Tuberculosis (TB) is an ancient scourge known to have plagued humans for centuries. The oldest known signs of this disease were found in Egyptian and Peruvian mummies[1,2] but a recent discovery in a Turkish *Homo erectus* fossil, an early human ancestor, indicates the occurrence of TB in humans for as long as 500,000 years.[3] This disease was also responsible for the "White Plague" that depopulated 17th–18th century Europe by 25%.[4] Even though the advent of vaccination, chemotherapy and improvement in health and sanitation infrastructure helped curb the spread of TB in mid-20th century, this respiratory disease has been staging a significant recurrence of late. Recent statistical projections have shown that *Mycobacterium tuberculosis* (MTB), the etiologic agent of TB, has infected one-third of the world's population and of these having latent TB, approximately 9 million cases will progress to active TB and an estimated two million people will die annually.[5]

The recent rise in the incidence of TB worldwide, especially in poor countries, can be attributed to a number of factors.[6,7] Chief among them is the ongoing Human Immunodeficiency Virus-Acquired Immunodeficiency Syndrome (HIV-AIDS) epidemic that compromises an infected person's immune system leading to development of active TB infection or reactivation of dormant TB. The HIV epidemic alone is thought to be responsible for one-third of the increase in global TB cases over the last five years.[8]

Moreover, the proliferation of the disease is also exacerbated by the manifestation of deadlier strains of *M. tuberculosis* impervious to current TB treatment. Strains of MTB resistant to at least two of the standard or first-line drugs used for current TB regimen are called multi-drug resistant TB (MDR-TB).[9] A far more deadly strain, called

extensively drug-resistant TB (XDR-TB), shows resistance to at least two of the first-line anti-TB drugs and is also immune to the second-line drugs.[9,10] XDR-TB received substantial news coverage in the past year, when a person believed to have this form of the disease travelled by plane between the North America and Europe and back, and in doing so exposing many people to possible infection. It was later determined that this individual had MDR-TB, not XDR-TB, but the media coverage of this event raised awareness of this important health issue.[11,12]

The very expensive and rigorous treatment process is a common obstacle that contributes to the increase in drug-resistant TB.[13] Specifically, this can be elaborated to the need for a sophisticated diagnostic process, length of obtaining test results and side effects attributed to TB drugs often lead TB patients to interrupt or abandon their course of treatment leading to development of aforementioned drug resistance. The treatment course for MDR-TB alone runs from 18–24 months and costs around 1400 times as much of normal TB which is estimated at approximately US$1979–$8196 while that for XDR-TB ranged from US$6843–$15579.[9] MDR-TB has an 80% mortality rate as observed in cases during an outbreak in North America and Europe from the late 1980's to early 1990's while 98% of the patients infected with the XDR strain died from the disease during an outbreak in Tugela Ferry, Kwazulu-Natal Province, South Africa from January 2005 to March 2006.[9,14-16]

1.2 Drugs Used to Treat Tuberculosis

Success in treating TB requires that the patient adhere to a regimen of multiple antibiotics over a period of several months. The standard therapy, referred to as Directly

Observed Treatment Short-Course (DOTS), involves monitoring the patient while a course of antibiotics is given.[17] This treatment, which if followed is usually successful for treating TB that is not drug-resistant, consists of the administration of isoniazid, rifampin, and pyrazinamide for two months (See Figure 1 for structures). After this period, isoniazid and rifampin are continued for another six months. In addition to these drugs, patients are also usually given ethambutol or streptomycin. When treating strains that are drug resistant, this regimen must be changed, and second-line antibiotics are often employed (any fluoroquinolone and one of the injectable drugs capreomycin, amikacin or kanamicin).[9,10]

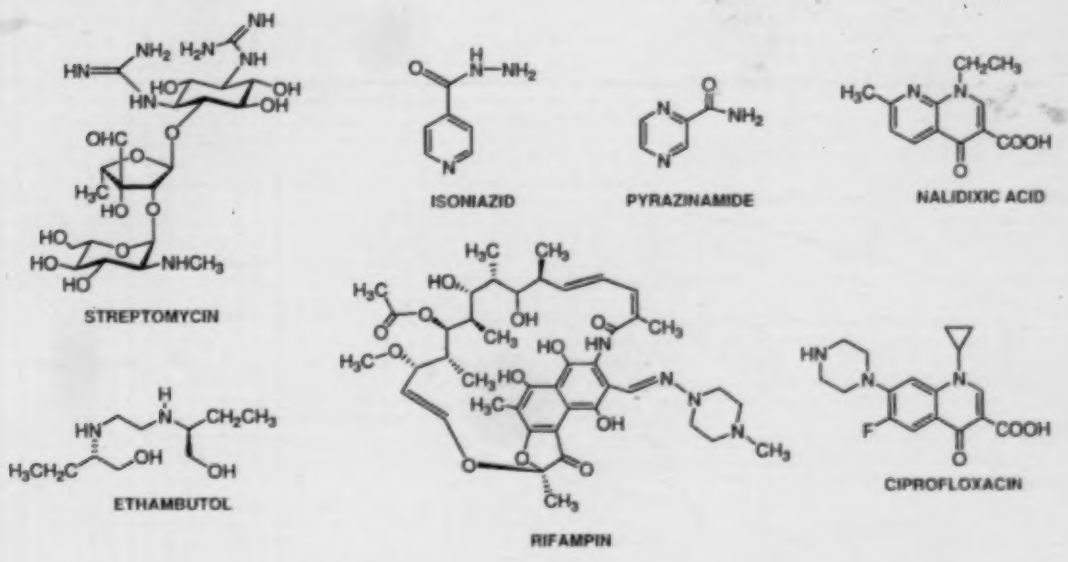


Figure 1. Structures of selected front-line anti-TB drugs in clinical use.

The mode of action of most front-line anti-TB drugs now is understood.[18,19] Isoniazid and ethambutol inhibit the assembly of the protective cell wall of the organism. In particular isoniazid prevents the formation of mycolic acids and ethambutol is an

arabinosyltransferase inhibitor (see section 1.4 for more information).[20,21] Rifampin binds to the β-subunit of DNA-dependent RNA polymerase, and therefore inhibits RNA synthesis.[22] Streptomycin, like other aminoglycoside antibiotics, inhibits protein synthesis by binding to the 30S subunit of the ribosome.[23] The only drug for which there is still ambiguity about its mode of action is pyrazinamide. It has been suggested that hydrolysis of the amide in the organism, thus giving pyrazinoic acid, leads to lethal acidification within the cell.[24] More recently, it has been proposed that the pyrazinoic acid liberated inhibits palmitic acid biosynthesis and hence interferes with lipid metabolism in the organism.[25]

1.3 Pathogenesis of Tuberculosis

There are several steps involved during infection of the host by *M. tuberculosis*.[26,27] The initial step involves inhalation of the infectious bacilli in the form of droplets or aerosols introduced into the atmosphere by another person. These droplet nuclei are generated by routine events such as talking, coughing, and sneezing, and can remain in the atmosphere for several hours. The inhaled bacteria in the lung are taken up by macrophages in the alveoli leading to a local inflammatory response, which in turn results in the influx of white blood cells called mononuclear cells from tissues supplying blood to the lung (Figure 2). This process leads to the formation of the granuloma or a tubercle, which is made up of infected tissues or macrophages and is encased by a mixture of immune cells and extracellular matrix components.

This is followed by the development of a complex structure enveloping the tubercle and the reduction in the number of blood vessels penetrating it. This fibrous

sheath acts as a barrier that helps to keep the mycobacteria in check and prevents its spread into other tissues. This microenvironment also allows the organism to survive and persist for a long period of time. Although the mechanism of persistence is still not fully understood, it is theorized that the mycobacteria adapts to a low oxygen, high carbon dioxide environment surrounded by hardened and dead cells.

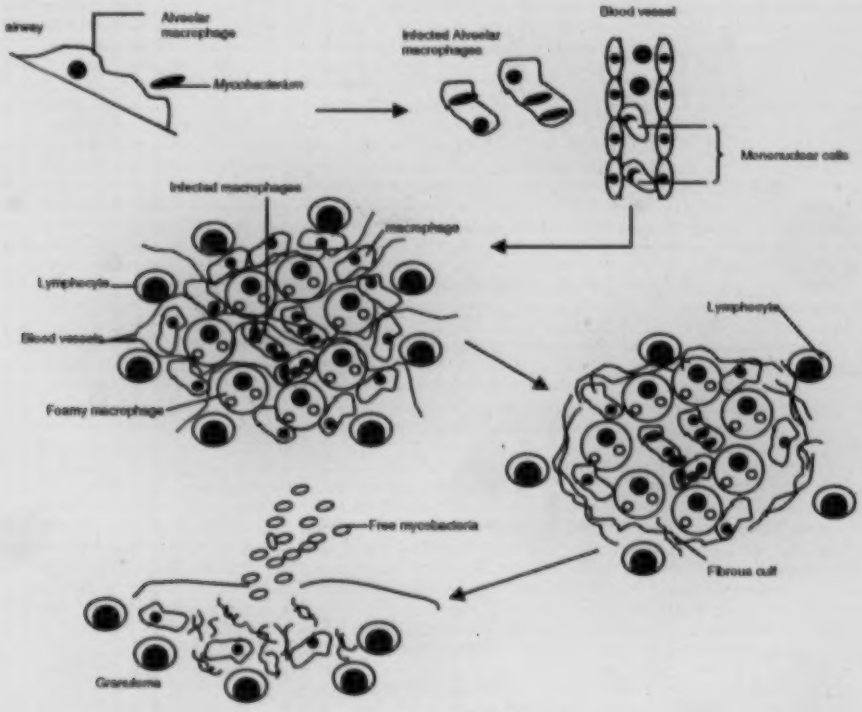


Figure 2. Pathogenesis of tuberculosis (Modified and adapted from Russell, 2007).[26]

The next stage of the infection process is dependent on the competency of the host's immune system. An immunocompromised individual will show acute signs of infection indicated by the spread of the mycobacteria to other cells. The diseased individual will typically suffer from fatigue, weight loss, and can spread the disease

through coughing. On the other hand, an immunocompetent individual will respond by suppressing the initial infection. The mycobacteria, however, will persist in the immunocompetent host, who is now considered latently infected.

The final infection stage involves the progression from latency to resumption of bacterial growth. The mechanism involved at this stage is also not fully understood but it is believed that any factor resulting in the failure of the host's immune system can trigger this activity. This process is characterized by breakdown of the tubercle causing it to rupture and release infectious bacilli into the airways; the individual then develops a cough that facilitates aerosol spread of infectious bacilli. Should the infected individual not receive treatment, these latter steps may lead to death.

Even though significant advances have been made in the study of TB, there are still fundamental problems that impede the development of a more effective anti-TB therapy. For example, there is a need for further elucidation of the basic biology of mycobacterial persistence in the host's macrophages during MTb infection.[22] In addition, most of the current anti-TB drugs have strong bactericidal effects but only exhibit weak sterilizing activity.[22] This means that these drugs act mostly on the actively growing mycobacterial cells but not on the slowly growing mycobacteria that persist or are dormant in the infected tissues.

The difficulty in eradicating persistent mycobacteria in the infected tissues is attributed to its heterogeneous nature that requires different types of drugs during TB chemotherapy (Figure 3).[22,28] Figure 3 shows the four types of bacterial populations and representative anti-TB drugs that are effective to a specific subpopulation.[22,28] It is known that in the early stages (two days) of TB chemotherapy, there is a very rapid eradication

of actively growing tubercle bacilli. This is followed by a longer period of slower extermination of the persistent bacilli by anti-TB drugs.[29] As mentioned earlier, the lengthy and rigorous process of the current treatment regimen, DOTS, although somewhat effective, often makes patient compliance very difficult. The development of new anti-TB drugs should therefore not only shorten the treatment regimen but should also be effective against both the persistent bacilli and drug-resistant strains. Although a number of the current anti-TB drugs have their mode of action as inhibitors of cell wall synthesis, there is still a need to perform basic research on the mycobacterial cell wall.

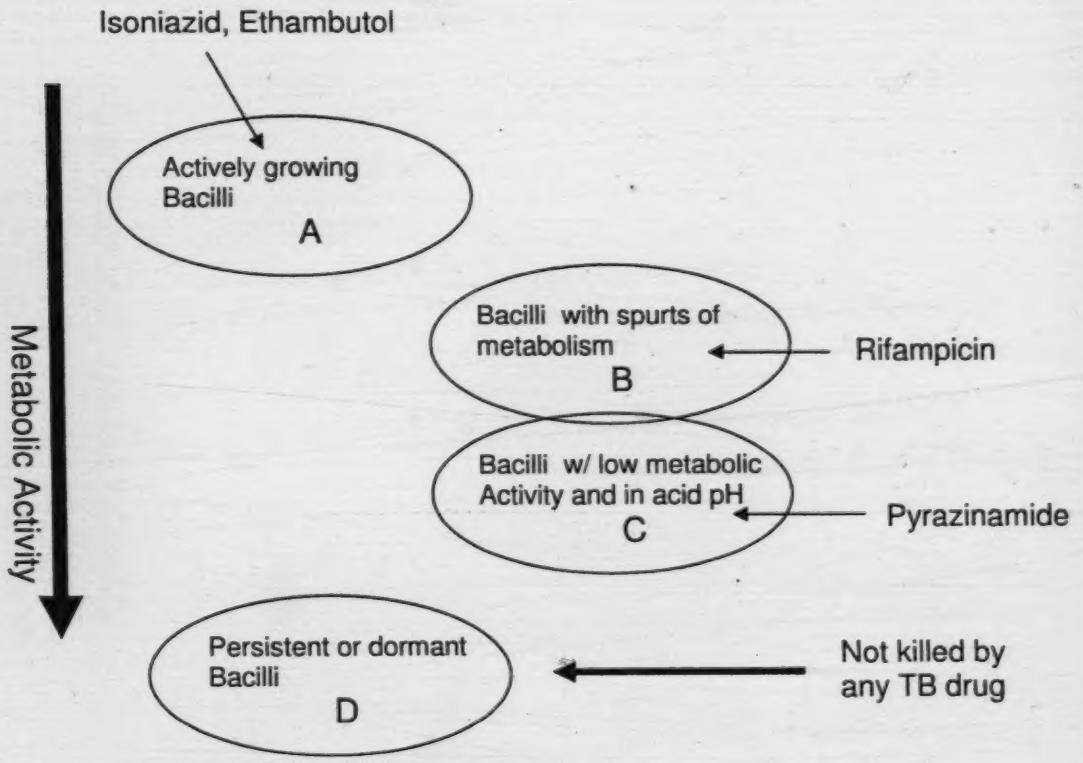


Figure 3. TB Drugs targeting distinct *M. tuberculosis* subpopulations (adapted from Zhang, 2005).[22]

1.4 Structure of the Mycobacterial Cell Wall

The mycobacterial cell wall is an important component of the organism and serves as a permeability barrier to the passage of antibiotics.[30] As described above, some of the front-line anti-TB drugs target the assembly of this structure. Given the importance of the cell wall to mycobacterial survival and pathogenicity there is much interest in obtaining a detailed picture of its structure.[31-36] It has been shown to have a number of unique structural motifs, and therefore the biosynthetic pathways by which cell wall components are assembled have attracted attention as the sites of action for new classes of anti-TB drugs.[37-43]

It should be pointed out that mycobacteria are a part of a larger group of bacteria, the actinomycetes, which all possess a similar cell wall. The actinomycetes most closely related to mycobacteria are the corynebacteria, norcardia and rhodococci. Thus, while the discussion below is focused on mycobacteria, similar structures are present in these other actinomycetes as well.[44,45]

The mycobacterial cell wall is a complex structure of proteins, lipids, glycolipids and polysaccharides.[46,47] There are four major components of cell wall, namely: 1) peptidoglycan, a porous layer between the cell wall and plasma membrane; 2) the mycolyl-arabinogalactan (mAG) complex attached to the peptidoglycan, which is composed of long chain fatty acids, mycolic acids, and the unique polysaccharide, arabinogalactan (AG), linked together by ester bonds; 3) lipoarabinomannan (LAM) and its truncated form lipomannan (LM), and, 4) "free" lipid species, called extractable lipids, that include glycolipids, phenolic glycolipids, glycopeptidolipids, and other chemical species.[48] A schematic diagram of the mycobacterial cell wall is shown in Figure 4.

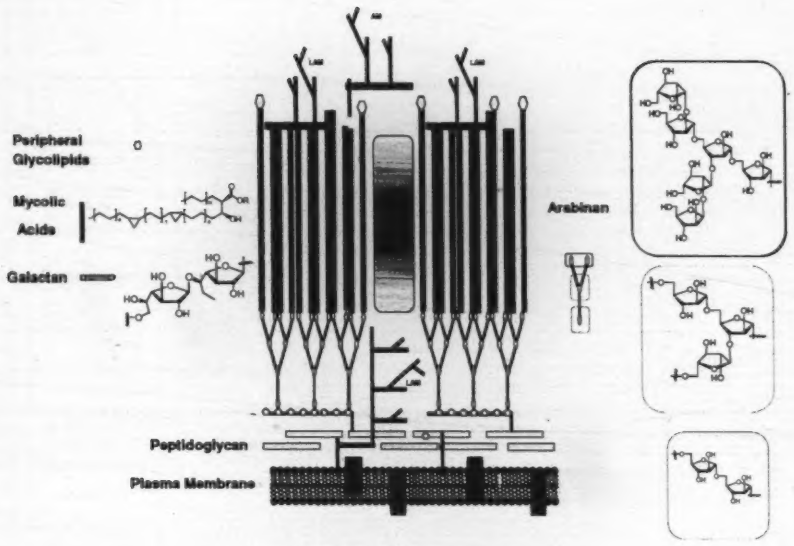


Figure 4. Schematic depiction of the mycobacterial cell wall.

1.4.1 Peptidoglycan

Peptidoglycan forms the backbone of the cell wall skeleton and consists of a polysaccharide chain formed from alternating units of *N*-acetylglucosamine and *N*-glycolylmuramic acid. The *N*-glycolylmuramic acid residues are further modified at O-3 with tetrapeptide side chains composed of L-alanyl-D-isoglutaminyl-meso-diaminopimelyl-D-alanine (Figure 5). Cross-linking between the peptidoglycan chains occurs either through two diaminopimelic acid residues or between D-alanine and diaminopimelic acid.[49-51]

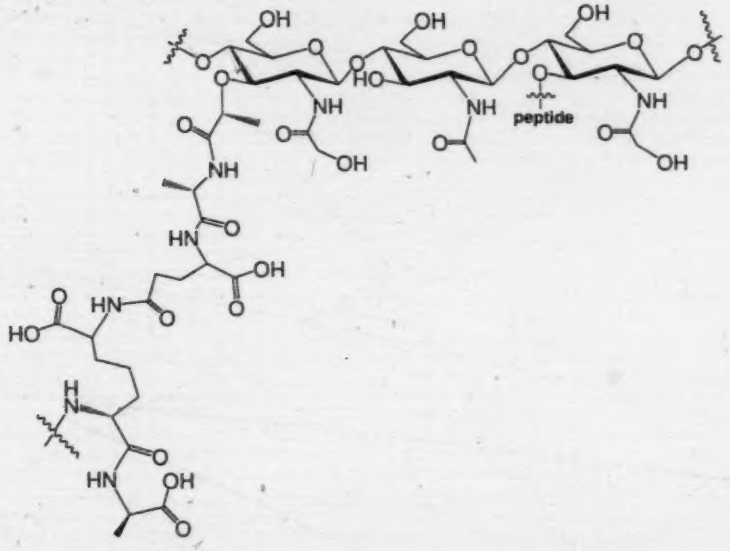


Figure 5. *N*-acetylglucosamine and *N*-glycolylmuramic acid linkages in the peptidoglycan.[45]

1.4.2 Mycolyl-Arabinogalactan-Peptidoglycan (mAGP) Complex

The mAGP complex is a lipidated polysaccharide composed of a polymer of galactofuranosyl and arabinofuranosyl residues covalently linked to peptidoglyan and is capped to its non-reducing terminus by hydrophobic mycolic acid esters (Figure 6). This complex is responsible for the impermeable nature of the mycobacterial cell wall, which leads to its resistance to the passage of antibiotics. It is also critical for mycobacterial survival and pathogenicity and its biosynthesis is targeted by the anti-TB drugs isoniazid and ethambutol.[18-21]

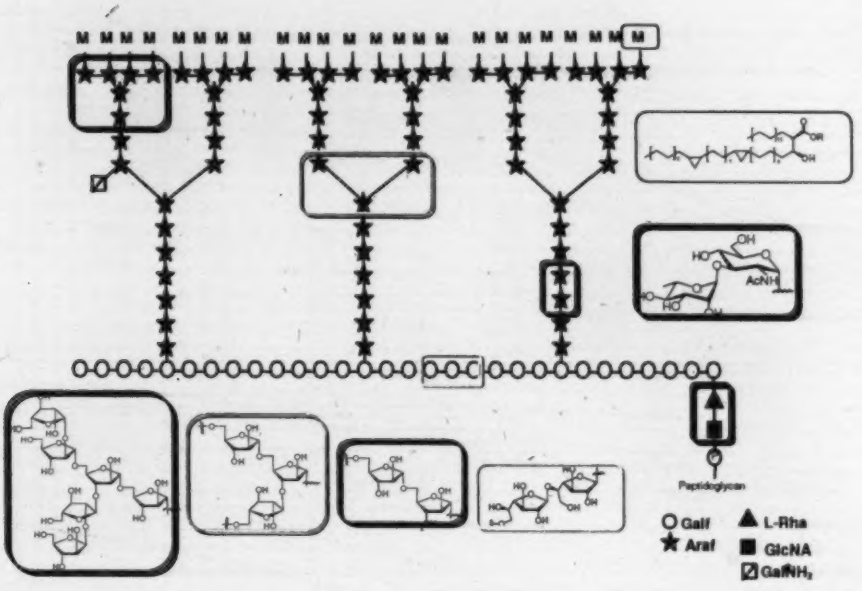


Figure 7. Schematic drawing of the structure of mycolyl-AG complex.

1.4.2.1 Arabinogalactan (AG)

The arabinogalactan (AG) is a unique structure comprised of D-galactofuranosyl (Gal*f*) and D-arabinofuranosyl (Ara*f*) monosaccharides.[52] This polysaccharide differs from most bacterial polysaccharides by having a few structural motifs instead of repeating units (Figure 7).[53] The AG is covalently bound to O-6 of muramic acid residues of peptidoglycan through a disaccharide phosphate linker unit, α-L-Rha*p*-(1→3)-α-D-Glc*p*NAc-PO_4. From the 4-position of the rhamnose residue is attached a linear chain of approximately 30 Gal*f* residues attached via alternating β-(1→5) and β-(1→6) linkages (Figure 7).

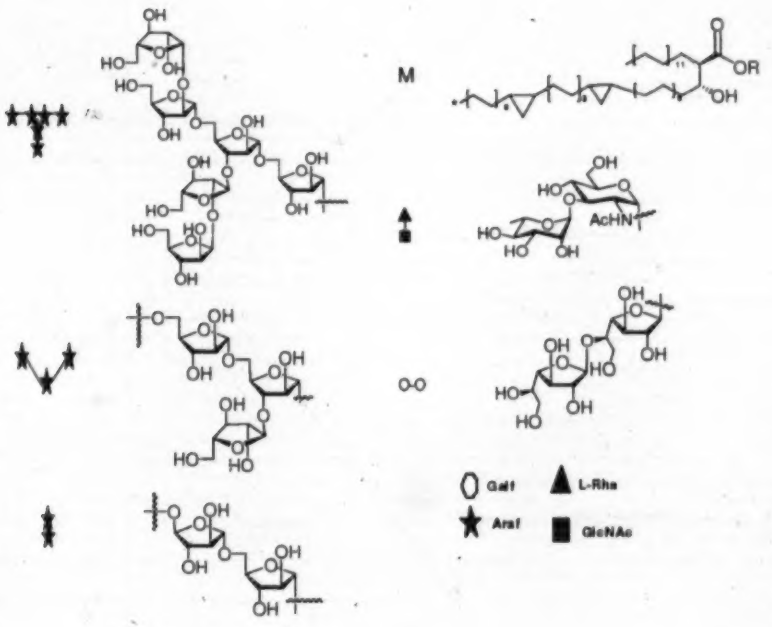


Figure 7. Structural motifs in mycobacterial mycolyl-AG complex.

Along the galactan chain, three arabinan chains[54] are attached each containing 31 Ara*f* residues arranged in a precise structure. These arabinan domains are attached to O-5 of the galactofuranosyl residues 8, 10 and 12 in the galactan core.

The arabinan domains consist of a core linear chain of ~14 α-(1→5) linked Ara*f* residues.[34] The terminal residue of this chain is branched at both at O-3 and O-5 with an α-Ara*f*-(1→5)-α-Ara*f* disaccharide, which is then capped with a branched hexasaccharide (Ara-6), containing two β-(1→2) linked Ara*f* residues (Figure 7). Recent findings indicate the presence of negatively charged succinyl residues[34] in addition to the positively charged $GalNH_2$[55] moeities. Either of these groups is attached to the 2-position

of the O-3, O-5-branched Ara*f* residue at the end of the linear inner arabinan chain. In these recent studies,[34] it was proposed that one-third of the arabinan chains were succinylated, one third contain galactosamine and the other third are unsubsituted at this residue. It was also shown that the arabinan chains that contain succinate esters are not esterified with mycolic acids, thus suggesting that introduction of this motif may inhibit the addition of these groups. This finding is consistent with earlier studies[56] that showed that only two-thirds of the arabinan domains are mycolated. The anti-TB drug ethambutol inhibits arabinan biosynthesis.[18,19,35]

1.4.2.2 Mycolic Acids

The impermeable and tightly packed outermost region of mycobacterial cell wall is primarily composed of mycolic acids, high molecular weight α-alkyl-β-hydroxy fatty acids containing 70–90 carbons. These linear carbon chains can be further elaborated with a number of additional structural motifs including double bonds, cyclopropanes, and oxygen-containing functional groups such as ketone, methoxy, and hydroxyl functionalities. Different mycobacterial species contain mycolic acids with different structures. As mentioned in the previous section, the mycolic acids are attached to two-thirds of the Ara-6 termini of the AG, and these groups are attached through ester linkages involving the primary hydroxyl groups.[57]

The three kinds of mycolic acids in the *M. tuberculosis* cell wall are the α-mycolates, methoxymycolates, and ketomycolates (Figure 8). Both the α-mycolates and methoxymycolates only have *cis*-cyclopropyl groups at the proximal position (closest to the hydroxyl group). In contrast, in the keto-mycolates a mixture of *cis*- and *trans*-

cyclopropyl groups are at the proximal position in an 83:17 ratio.[58] The short α-chain of all three mycolates contain 24 and 26 carbons in an approximately 1:9 ratio, with negligible amounts of 22 carbon chains. The longer (mero) chain in the methoxymycolates and ketomycolates has longer chains than those present in the α-mycolates. The total carbon numbers for the α, methoxy, and keto mycolates are 76–82, 83–90, and 84–89, respectively.[59] The mero chain is proposed to be stabilized by parallel packing between the hydrocarbon chains with the presence of *cis* double bonds and *cis* cyclopropane structures modulating the tight packing. The amount of mycolic acid containing *trans* substituents defines the flexibility of the mycobacterial cell wall and its permeability towards hydrophobic antibiotics.[60] Isoniazid, which is used to treat TB, inhibits the biosynthesis of the mycolic acids.[20]

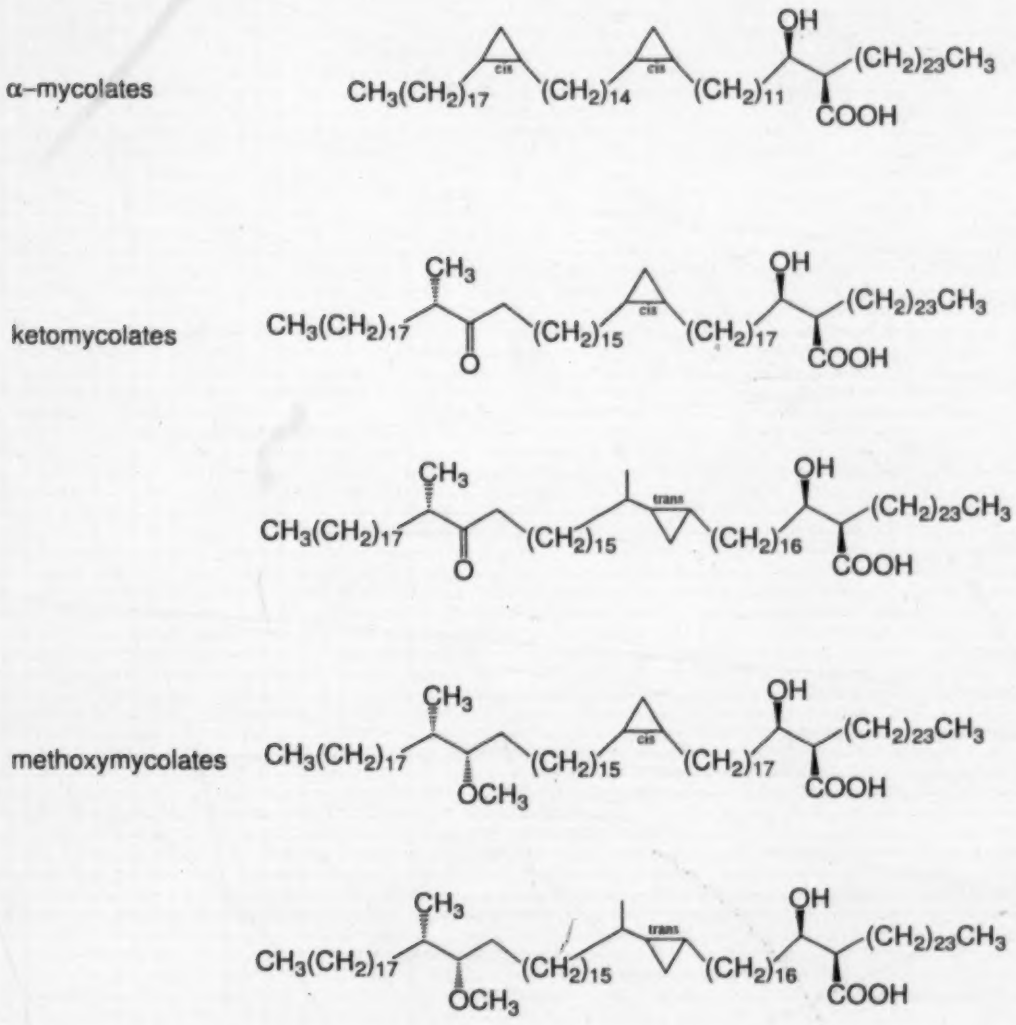


Figure 8. Representative structures of mycolic acids.

1.4.3 Lipoarabinomannan (LAM) and Lipomannan (LM)

The lipoarabinomannan (LAM) is a polymer with a mannopyranosyl core and arabinofuranosyl side chain residues while the lipomannan (LM) is its analog without the arabinan (Figure 9). These polysaccharides are located in between the AG branch chains

and are attached noncovalently to the cytoplasmic membrane through the lipid portion of a phosphatidylinositol linker.[30] Mannopyranosyl residues having α-(1→6) linkages, half of which contain an α-(1→2) mannopyranosyl branch, comprise the mannan core of these polysaccharides. Structural studies of LAM showed that there are two distinct arabinan motifs situated at its terminal end: the branched hexaarabinofuranosides (Ara-6) identical to that in AG, and a linear β-D-Ara*f*-(1→2)-α-D-Ara*f*-(1→5)-α-D-Ara*f*-(1→5)-α-D-Ara*f*-tetrasaccharide (Ara-4).

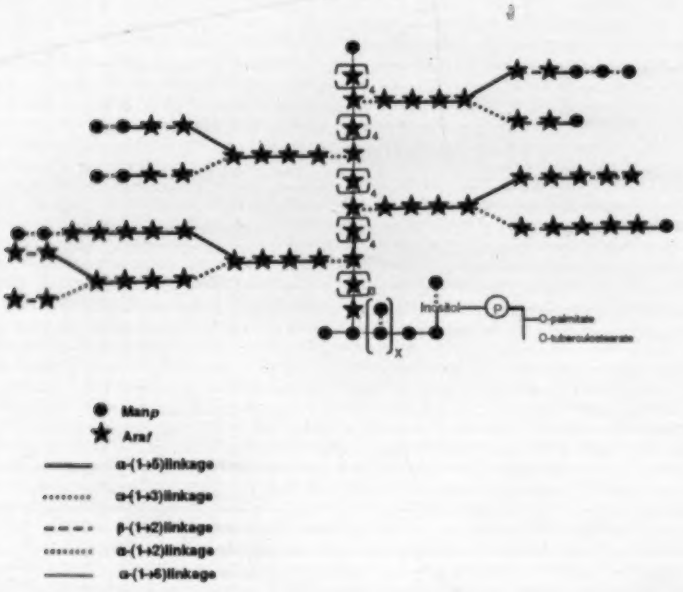


Figure 9. Schematic representation of mycobacterial lipoarabinomannan (LAM).

The arabinan domain of LAM contains fewer branch points than that in AG and there are no mycolic acids esterified to the terminal hexasaccharide motif. However, the β-D-Ara*f*-residues in Ara-6 and Ara-4 are capped with different motifs, which depend on

the mycobacterial species. In *M. tuberculosis*, these capping motifs are short α-(1→2)-linked mannopyranosyl residues, resulting in a structure called ManLAM.[61,62] Another capping motif present in *M. tuberculosis* is a 5-thiomethylxylofuranose residue, which is α-(1→4)-linked to a mannopyranose residue.[63] In *M. smegmatis*, these capping motifs are inositol phosphate moieties (yielding PI-LAM)[64,65] and in some species (e.g., *M. chelonae*) there are no capping motifs, resulting in a structure called AraLAM.[66]

1.4.4 Extractable lipids

Extractable lipids are lipids and glycolipids that are non-covalently associated with mycolic acids located at the periphery of the cell wall complex. The lipid portion in these glycolipids intercalate into the mycolic acids, and the oligosaccharide resides at the outermost part of the cell wall interact with the host.[56,67]

Representative examples are shown in Figure 10. These molecules are believed to be important modulators of the immune response arising from infection by mycobacteria, but this process is not well understood.[68]

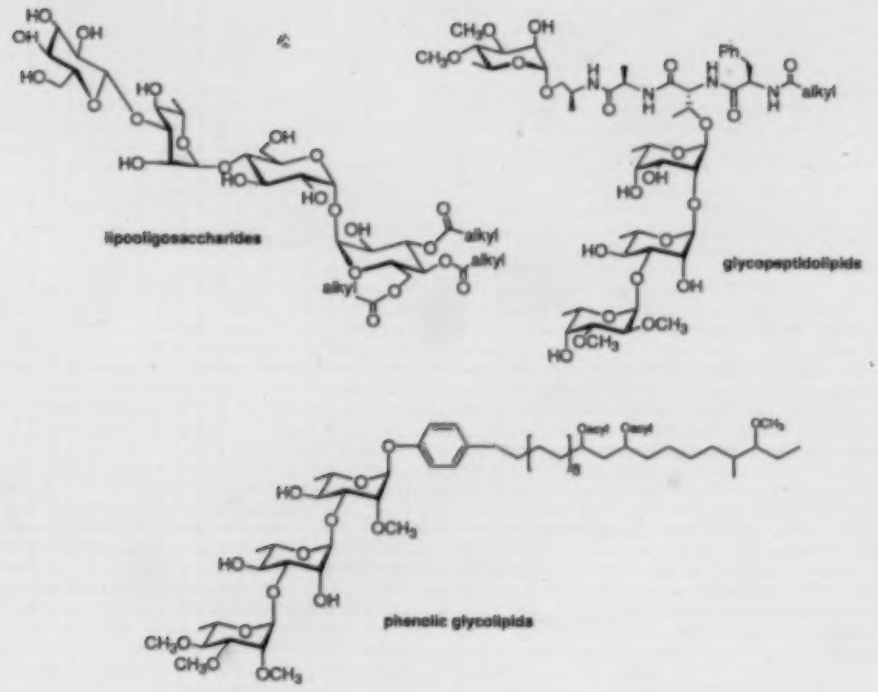


Figure 10. Representative examples of extractable lipids.

1.5 Biosynthesis of the Mycolyl-Arabinogalactan-Peptidoglycan Complex (mAGP)

A current thrust in *M. tuberculosis* research is the identification of novel targets for the action of new anti-TB drugs.[22] A strong emphasis has been placed on the study of biosynthesis of the major component of cell wall, mAGP, which is essential to the microorganism's viability.[22] The identification and characterization of the enzymes involved in cell wall synthesis is therefore important and has received increasing attention in recent years.[38,45,69,70]

The general features of mAG are now well established and over the past several years many of the enzymes involved in the process have been identified.[32,38,71-77] The

proposed biosynthetic pathway for mAGP (Figure 11) starts with the transfer of a GlcNAc-1-phosphate from UDP-GlcNAc to prenyl phosphate (C_{50}-P);[78] the enzyme proposed to catalyze this transfer in *M. tuberculosis* H37Rv is encoded by the *Rv1302* gene.[79] The formation of GlcNAc-P-P-polyprenyl is followed by the addition of rhamnose (Rha) from dTDP-Rha, catalyzed by the rhamnosyl transferase Wbbl (*Rv3265c* gene), leading to the formation of the linker unit of AG (see Figure 4).[32,80]

Once the linker unit is produced, the galactan domain is formed by the sequential addition of Gal*f* residues. This process involves galactofuranosyltransferases that use UDP-galactofuranose (UDP-Gal*f*) as the donor species.[74-76,81,82] The synthesis of UDP-Gal*f* is achieved by the conversion of UDP-glucose to UDP-galactopyranose (UDP-Gal*p*) catalyzed by UDP-galactopyranose epimerase encoded in *M. tuberculosis* by the gene *Rv3634*. UDP-Gal*p* is then converted to UDP-Gal*f* catalyzed by the enzyme UDP-galactopyranose mutase that is encoded by *Rv3809c*.[83]

At the time my thesis work was started, it had been suggested that all of the Gal*f* residues were added by a single bifunctional galactofuranosyltransferase, then called glfT, which was encoded by *Rv3808c* gene.[75] This enzyme had been demonstrated to be bifunctional and capable of adding alternating β (1→5) and β (1→6) linked Gal*f* residues.

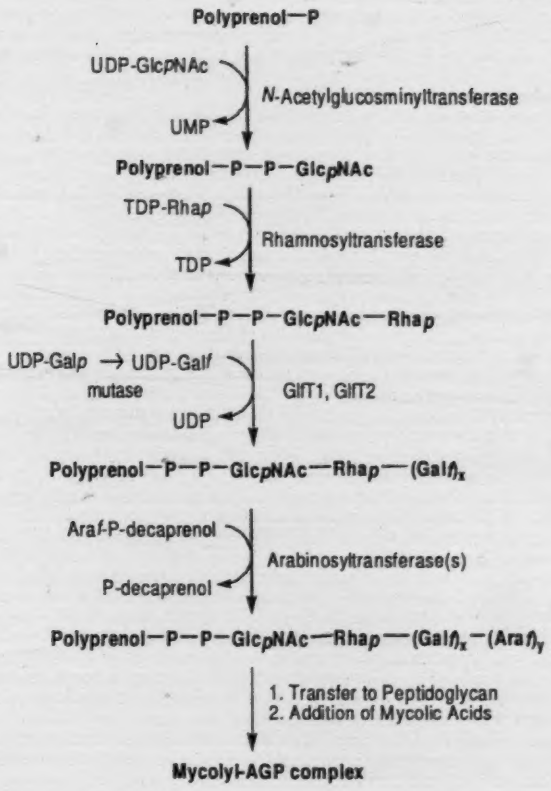


Figure 11. Biosynthetic pathway for mAGP complex.

As outlined in the next chapter, the compounds I synthesized were used to probe the specificity of glfT (now called GlfT2) and to help demonstrate that a second galactofuranosyl transferase (GlfT1, encoded by the *Rv3782* gene) is also involved in the process. Thus, as discussed in more detail below, my work was instrumental in establishing that galactan assembly proceeds by the action of two enzymes, GlfT1 and GlfT2, both of which have dual activity.[84]

The subsequent addition of arabinofuranose (Ara*f*) residues to the lipid-linked galactan intermediate acceptor involves the sugar donor β-D-arabinofuranosyl-1-

monophosphoryldecaprenol (DPA),[85] which is used by a number of arabinosyltransferases to produce the α-(1→5)-, α-(1→3)- and β-(1→2)-Araf linkages of the AG.[85-88]

The number and identity of these arabinosyltransferases remain unclear. The proteins encoded by the *embA* and *embB* genes, EmbA and EmbB, have been reported to function as arabinosyltransferases involved in the AG synthesis in *M. tuberculosis*. These enzymes are ethambutol-sensitive and hence believed to be the target of this anti-TB drug.[72] In addition, it was identified that the enzyme AftA,[71] (encoded by the *Rv3792* gene) catalyzes the addition of the first Araf residue from DPA to the galactan domain of AG. This transferase is ethambutol-insensitive, as is AftB (the protein product of the *Rv3805c* gene), which adds the β-Araf residues to the AG.[77]

It is likely that a number of other arabinosyltransferases are involved in the assembly of this polymer, but at this point their identity remains unknown. Once assembled, the AG-lipid intermediate is then ligated to peptidoglycan.[69] This process involves the transphosphorylation of the terminal GlcNAc-1-P of the linker unit of the AG polymer from its polyprenyl-P carrier to the peptidoglycan with release of polyprenyl phosphate. Finally, the mycolic acids are added to the structure to generate the fully formed mAG complex. The details of these final steps remain poorly understood.

1.6 Cell Wall Core Galactan as a Potential Target for the Development of New Anti-TB Drugs

The importance of the galactan to mycobacterial growth and viability can be rationalized given the role it plays in linking the peptidoglycan and mycolic acids. The

mycolic acids are essential in providing the organism with protection from its environment and anything that prevents these lipids from being expressed on the outer surface of the mycobacterial cell will lead to death.[33,89,90] Support for the general approach of targeting components that link the mycolic acid to the cell wall comes from studies showing that the anti-TB drug, ethambutol, prevents the synthesis or mycobacterial arabinan.[72]

As detailed above, the arabinan, together with the galactan, serve as the structure that links the mycolic acids to the cell wall. Hence, the enzymes catalyzing the formation of Gal*f*-linkages in *M. tuberculosis* are therefore viable targets in anti-TB drug discovery. Further support for this approach is the discovery that mycobacteria that are unable to synthesize the galactan are not viable.[31]

Despite the importance of the galactan to mycobacterial survival, none of the currently used anti-TB have been demonstrated to inhibit its biosynthesis.[31] Although targeting the enzyme that produces UDP-Gal*f* from UDP-Gal*p* (UDP-Gal*p* mutase) is a viable approach, we have chosen to focus on the galactofuranosyltransferases that employ this sugar nucleotide. In the following section, I will discuss the enzymes involved in the process and their relationship to my thesis work. This section is presented from a chronological perspective.

1.6.1 UDP-Galactofuranosyltransferase 2 (GlfT2)

When I started my thesis work, two papers had just appeared in which analysis of the genome from *M. tuberculosis* H37Rv led to the identification of the gene (*Rv3808c*) encoding for a protein called glfT,[75] a 68 kD protein that transfers Gal*f* residues from

UDP-galactofuranose to the growing galactan chain yielding an elongated product (Figure 12). More recently this enzyme has been renamed GlfT2.

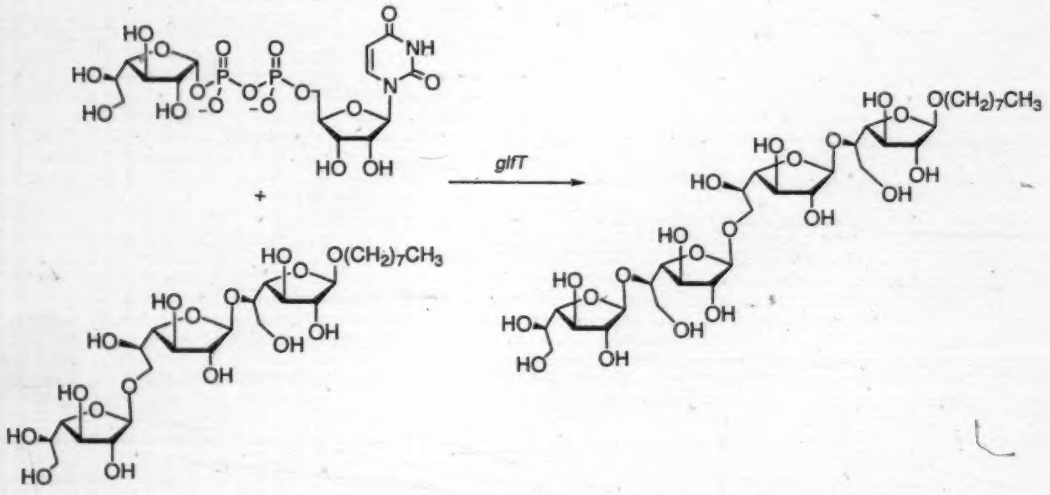


Figure 12. Representative reaction catalyzed by GlfT2.

This enzyme uses UDP-Gal*f* as the donor substrate, which has the α-stereochemistry, and provides products with β-stereochemistry. Therefore, the enzyme has an inverting mechanism. Glycosyltransferases that proceed with inversion are believed to involve a direct S_N2 displacement of the nucleoside diphosphate by the acceptor alcohol.[91,92] The displacement reaction is facilitated by a general base on the enzyme, which deprotonates the incoming nucleophile (acceptor) (Figure 13).

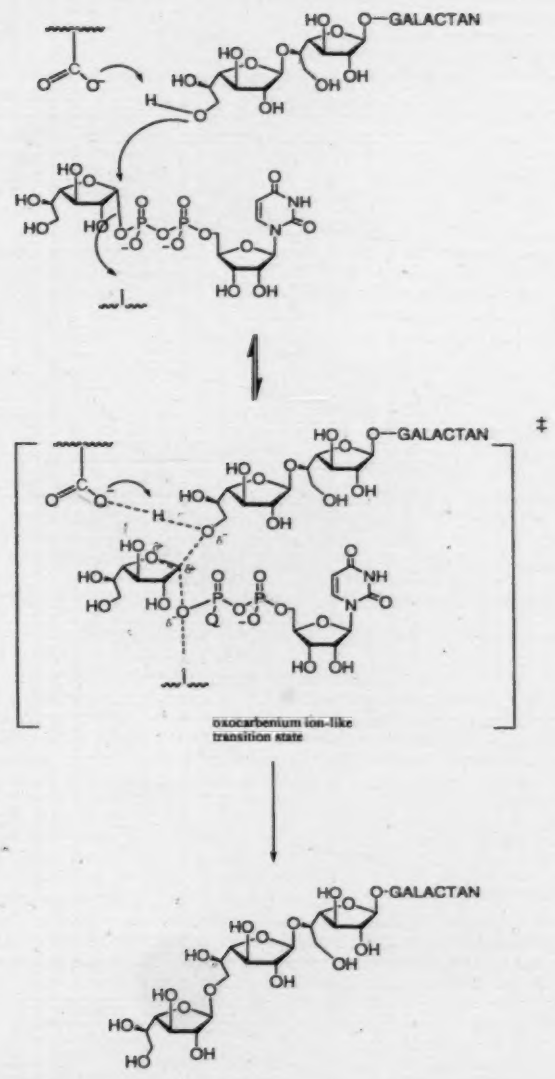


Figure 13 . Proposed mechanism of inverting GlfT2.

In addition, an acidic residue on the protein, or metal ion, often complexes with the nucleoside diphosphate leaving group to facilitate C–O bond cleavage. This model is supported by structural and mechanistic investigations of other inverting glycosyltransferases[91] as such studies have not, to date, been carried out on GlfT2.

In the genome, *Rv3808c* overlaps, by four nucleotide bases, with *Rv3809c*, the gene encoding for UDP-Gal*p* mutase, the enzyme that converts UDP-Gal*p* into UDP-Gal*f* (Figure 14). In earlier work, Besra and coworkers demonstrated that overexpression of GlfT2 in both *M. smegmatis* and *E. coli* led to membrane preparations that could be used to develop a radiochemical assay for its activity. A limitation in assaying galactofuranosyltransferases is that the donor substrate UDP-Gal*f* is not commercially available, and was,[93-97] until recently,[98] difficult to synthesize. Hence, in the assay developed by Besra, UDP-Gal*f* was generated in the assay mixture from commercial UDP-Gal*p*, using mutase that was endogenous in the membrane preparation.

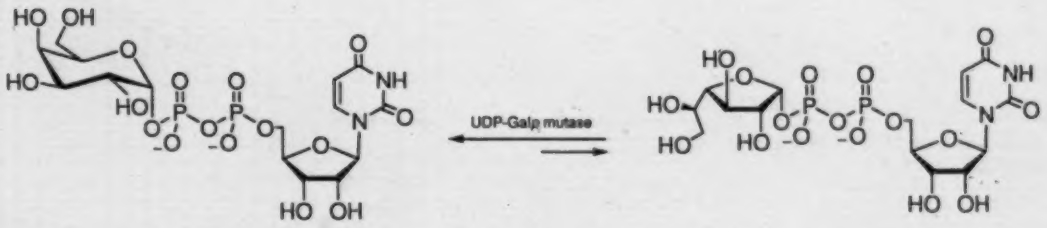


Figure 14 . Reaction catalyzed by UDP-Gal*p* mutase.

Using this assay with synthetic oligosaccharide substrates, the enzyme was shown to possess dual β-(1→5) and β-(1→6)-galactofuranosyltransferase activity and hence the enzyme is one of a relatively small number of glycosyltransferases that are bifunctional.[74] Although these studies showed the bifunctionality of GlfT2, pure protein was not obtained and thus it was of interest to express and purify this enzyme and evaluate its substrate specificity. When we started this study, no one had recombinantly expressed GlfT2, nor had detailed kinetic studies been done on the protein. In conjunction with the

work described in this thesis, our group reported the first high-level expression and purification of GlfT2 via expression of the *Rv3808c* gene in *E. coli* C41(DE3) cells.

The bulk of my thesis work involved the synthesis of a panel of oligosaccharides (**1**–**8**, Figure 15), which were used to characterize recombinant GlfT2 and to develop an assay for its activity. In the next chapter, a detailed discussion of the approaches taken toward their synthesis is provided. An efficient one-pot method for the synthesis of compounds **5** and **6** was also developed. Following the synthesis of these compounds, a detailed evaluation of the substrate specificity of *glfT* was carried out by a collaborator in the lab of Dr. Natisha Rose. The previous studies by Besra and coworkers used disaccharide analogs (analogous to **1** and **2**) to probe the specificity of GlfT2, but one goal of our work was to explore larger substrates to determine the effect of chain length on the catalytic activity. As further probes for the enzyme, we also prepared disaccharide analogs **3** and **4** where the nonreducing end lacks the hydroxymethyl group, and tetrasaccharides **7** and **8**.

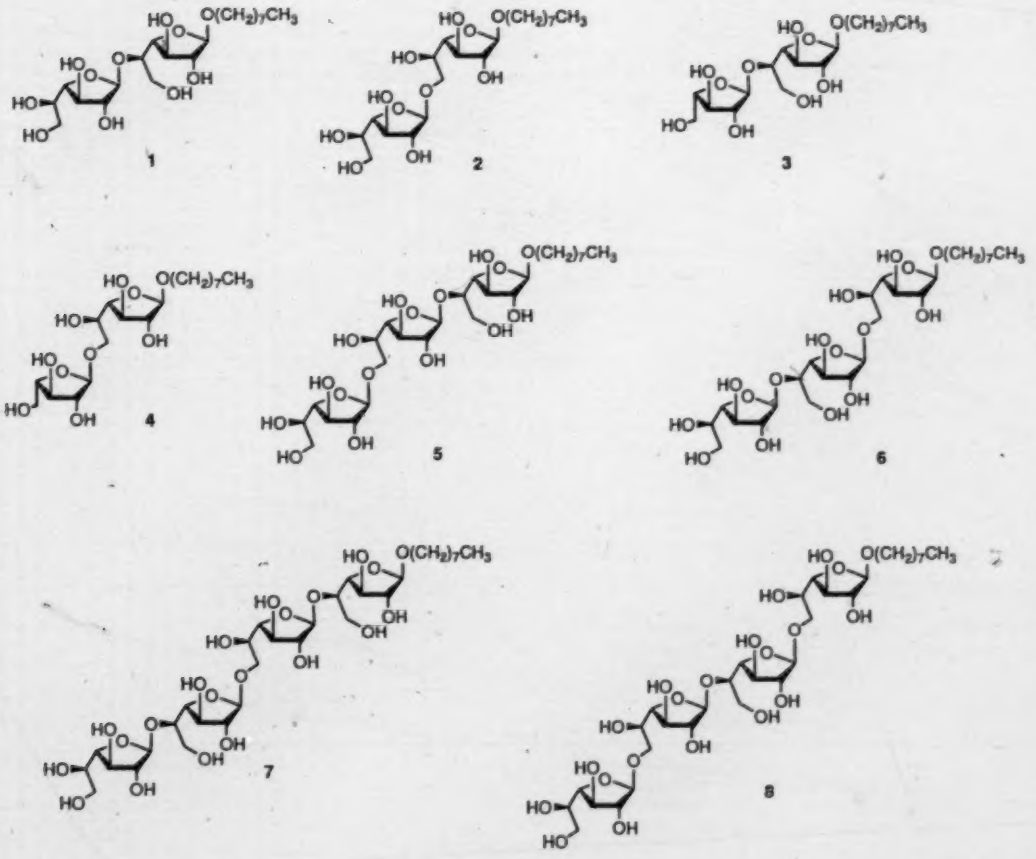


Figure 15. Synthesized compounds used to probe specificity of GlfT2.

1.6.2 UDP-Galactofuranosyltransferase 1 (GlfT1)

As this work proceeded, we began to think about the role of GlfT2 in the assembly of the entire galactan. Based on the glycosyl linkages present in the galactan chain (Figure 16) and the results of kinetic studies done on GlfT2 (see next chapter), we proposed that three additional galactosyltranferases, or at least three additional catalytic activities, would be involved in the galactan polymerization.[76]

We proposed that one enzyme would add the first, β-(1→4)-linked Gal*f* residue, to the Rha moiety, a second enzyme would add the second Gal*f* residue and a third would add the third Gal*f* residue. This process would lead to a pentasaccharide structure, which, we proposed, was a substrate for GlfT2 leading to full-length polymer synthesis.

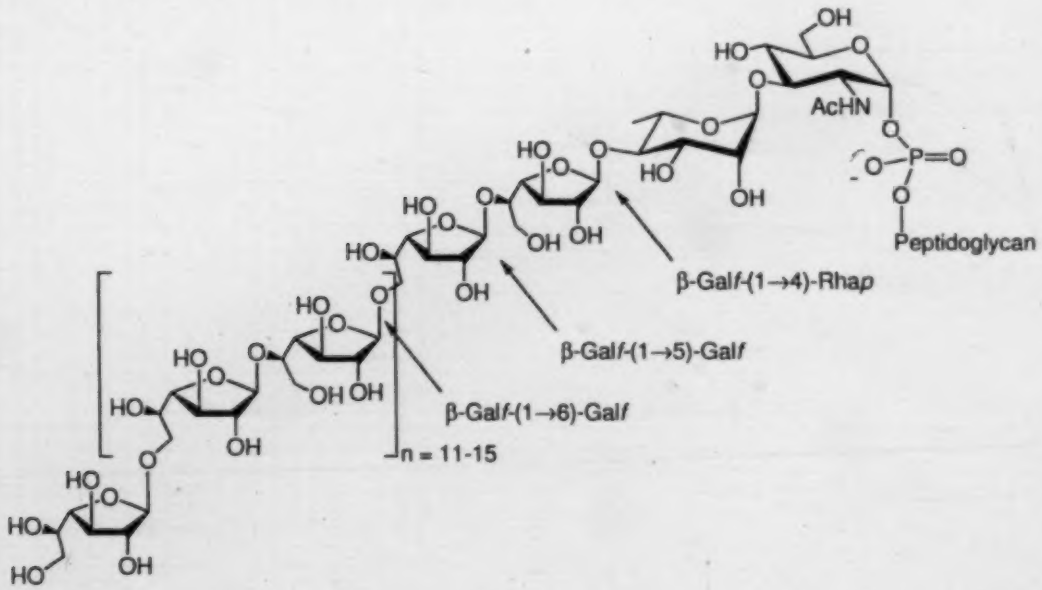


Figure 16. Galactofuranosyl linkages present in the galactan.

To test this hypothesis, we synthesized additional substrate analogs having the linker unit (**9–12**) as shown in Figure 17. During the course of this work, a paper by Mikušová, Brennan and co-workers appeared, identifying a gene, *Rv3782*,[82] which coded for a 307 amino acid residue protein that was proposed to be a galactosyltransferase involved in the first stages of galactan synthesis. Time-course experiments suggested

that, like GlfT2, the enzyme encoded by *Rv3782* was also bifunctional and that it was responsible for the introduction of the first two Gal*f* residues into the galactan.

With compounds containing the α-L-Rha*p*-(1→3)-α-D-Glc*p*NAc linker unit (**9–12**) in hand, we were well placed to enter into a collaborative project with the aforementioned research group to establish the role of these two enzymes in galactan assembly. As outlined in the next chapter, this collaboration further verified that the *Rv3782* gene encodes for a galactofuranosyltransferase, which is involved in the early stages of galactan synthesis, producing a molecule containing two Gal*f* residues. The product is then a substrate for GlfT2, which catalyzes the subsequent polymerization events leading to the formation of the bulk of the galactan chain containing alternating β-(1→5) and β-(1→6) Gal*f* residues.

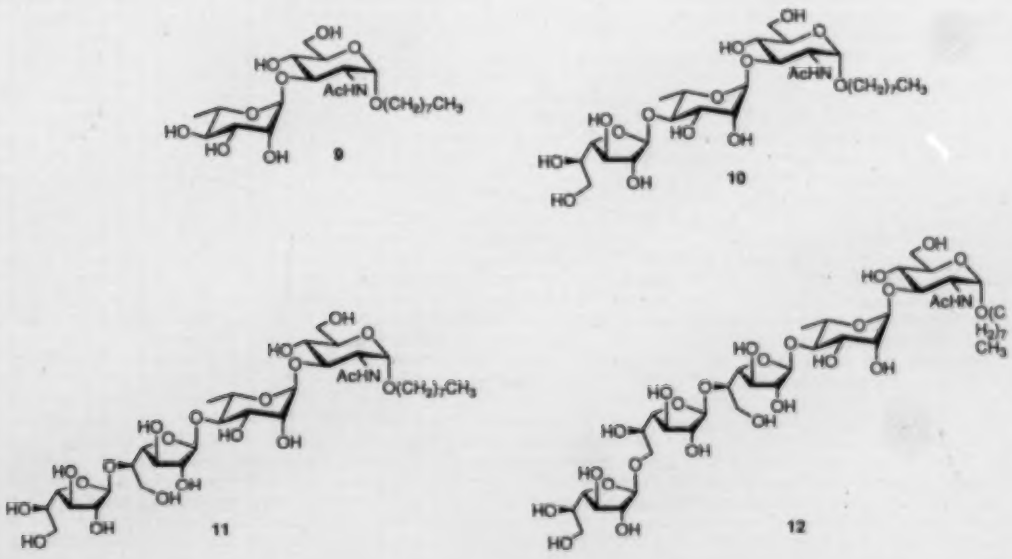


Figure 17. Compounds synthesized containing the linker unit.

1.7 Project Goals

The main objectives of this research were:

(1) To design and synthesize galactofuranose containing acceptor substrates for mycobacterial galactofuranosyltransferases (**1–12**) and,

(2) To evaluate the substrate specificity of GlfT1 and GlfT2 using the synthesized acceptor substrates.

CHAPTER 2

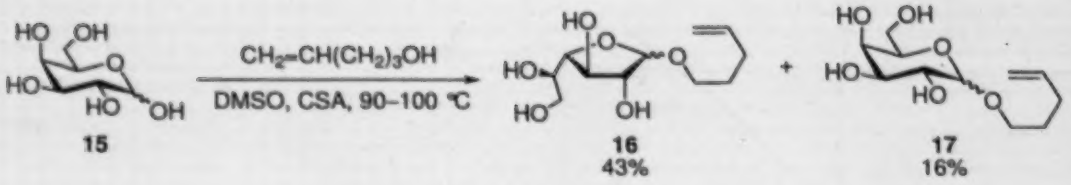
RESULTS AND DISCUSSION

2.1 Introduction – Methods for Accessing Galactofuranose Derivatives.

The first challenge for accessing the target compounds was to prepare galactose building blocks in the furanose form. A number of methods have been reported for carrying out this conversion, including kinetically-controlled Fischer glycosylation,[99-101] high temperature acylation,[102] cyclization of dithioacetals,[103] or open-chain *S,O*-acetals,[104] the ring opening of 1,4-anhydrogalactopyranose derivatives,[105] and the formation of 1,2:5:6-di-*O*-isopropylidene derivatives.[106-108] These are summarized briefly below.

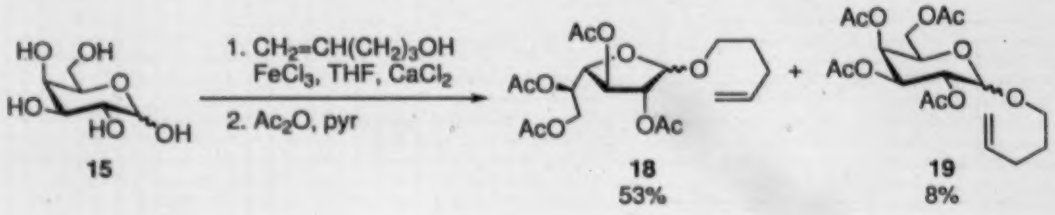
2.1.1 Fischer Glycosylation.

It is well established that when hexoses are treated with alcohols under Fischer glycosylation conditions (acid in an alcoholic solvent), furanosides are formed initially, which are then converted to their pyranoside forms.[109] Furanosides are therefore the kinetic products of this reaction and are often formed in excellent yields as a mixture of anomers, if proper conditions are chosen. D-Galactose was reported to be converted to a mixture of anomeric methyl furanosides via a ferric chloride catalyzed Fischer glycosylation.[99] Fraser-Reid and coworkers also reported a Fischer glycosidation of D-galactose under kinetic conditions using *n*-pentenyl alcohol and DMSO as co-solvent at 90–100 °C for six hours to afford *n*-pentenyl galactofuranoside (**16**) as a mixture of anomers (Scheme 1).[100] The reaction also gave the corresponding anomeric mixture of pyranosides (**17**), albeit in poor yield. A modified method for the preparation of *n*-pentenyl furanosides was later introduced wherein glycosylation of 4-penten-1-ol with D-



Scheme 1. Fischer glycosidation of D-galactose.[100]

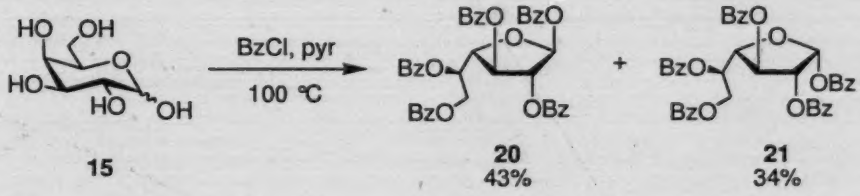
galactose was done in tetrahydrofuran by using ferric chloride as the promoter and calcium chloride as an additive. The reaction afforded, after 54 hours at room temperature and *in situ* acetylation, a mixture of furanosides (**18**) and the corresponding pyranosides (**19**) with a net preference for the β-furanoside and α-pyranoside derivatives (Scheme 2).[101] The favorable formation of the furanoside products over the thermodynamically more stable pyranosides was attributed to the complexation of the furanoside forms by Ca^{+2} ion.[110]



Scheme 2. Modified synthesis of *n*-pentenyl galactofuranosides.[3]

2.1.2 High Temperature Acylation.

The first synthesis of perbenzoylated β-D-galactofuranoside (**20**) and α-D-galactofuranoside (**21**) was discovered serendipitously through the attempted preparation of 1,2,3,4,5-penta-*O*-benzoyl-β-D-galactopyranose.[102] Treatment of D-galactose with benzoyl chloride and pyridine at 100 °C resulted in the formation of **20** and **21** instead of the target galactopyranoside product (Scheme 3). The structures of these compounds were unambiguously characterized by ^{1}H NMR and ^{13}C NMR spectroscopy and the observed fragmentation patterns obtained in the mass spectra, which were in agreement with a previously proposed patterns for similar acetylated derivatives.[111]

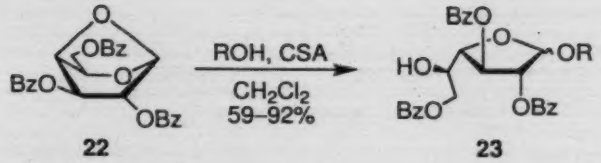


15 **20** 43% **21** 34%

Scheme 3. High temperature benzoylation of D-galactose.[102]

2.1.3 Regioselective Opening of 1,4-Anhydrogalactopyranose Derivatives.

An unconventional approach to the synthesis of galactofuranosides from galactopyranose has also been reported.[105] This method involves the reaction of protected 1,4-anhydrogalactopyranose derivatives with alcohols in the presence of camphorsulfonic acid (CSA) for 3–4 days, which results in the regioselective ring opening of the bicyclic system to yield the desired galactofuranosides (Scheme 4).

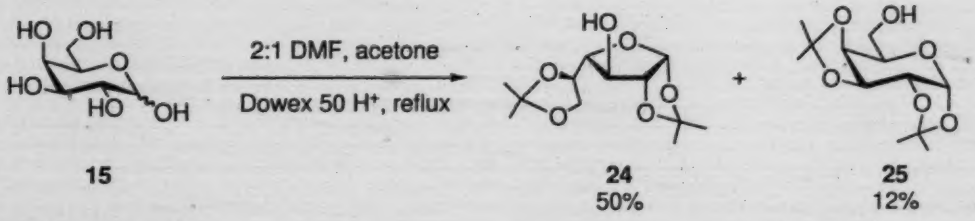


22 23

Scheme 4. Ring opening of 1,4-anhydro-2,3,6-tri-*O*-benzoyl-β-D-galactose.[105]

2.1.4 Synthesis of Di-*O*-isopropylidene Derivatives.

1,2:5,6-Di-*O*-isopropylidene-α-D-galactofuranose (**24**) has found limited use in synthetic carbohydrate chemistry due to the difficulties inherent in its preparation. Compound **24** can be prepared from D-galactose in three or six steps,[112,113] or from D-galactose in a maximum 22% overall yield.[106] An improved method for the preparation from D-galactose, requiring the use of zeolite catalyst, significantly increased product selectivity up to 40% of furanose diketal **24** but also gave 20% of pyranose diketal **25**.[108] A recent paper reported an improved synthesis of **24** by carrying out the reaction in a mixture of DMF and acetone, and employing a solid-supported catalyst, which gave a 50% yield of the product (Scheme 5).[107]

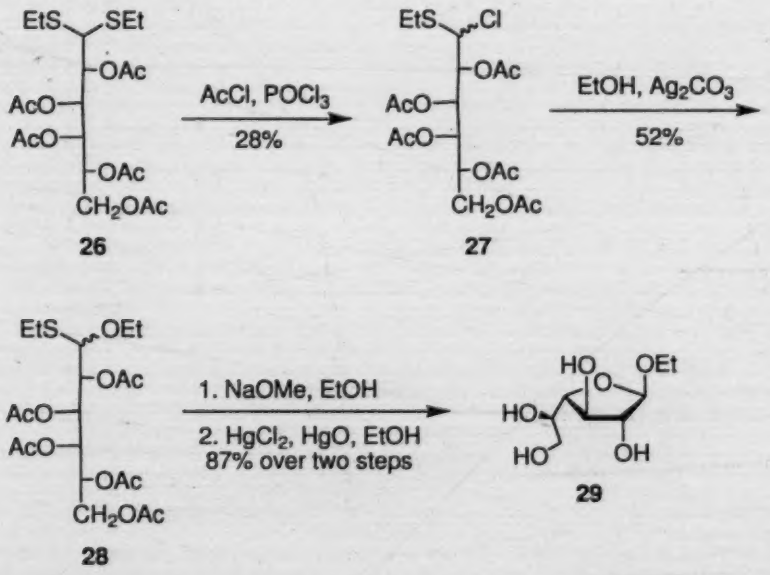


15 24 50% 25 12%

Scheme 5. Synthesis of 1,2:5,6-di-*O*-isopropylidene-α-D-galactofuranose, **24**.[107]

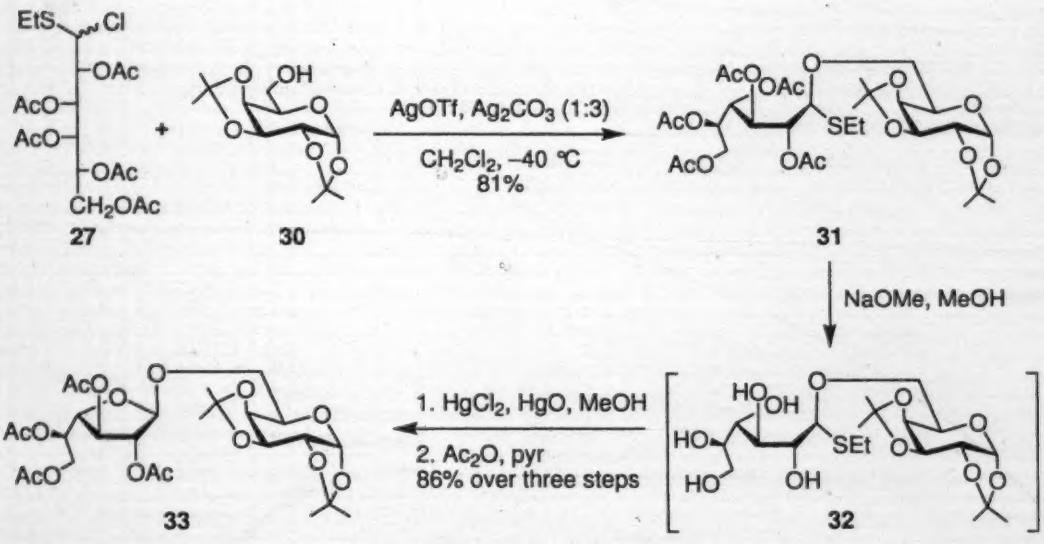
2.1.5 Cyclization of Dithioacetals.

Acyclic glycosyl dithioacetals were first prepared in the early 1890's[114] and have been used as precursors in the synthesis of glycosyl furanosides. The conversion of sugar dithioacetals to furanosides upon treatment with $HgCl_2$ and HgO in the presence of various aliphatic alcohols has been reported.[115,116] A modification of this procedure was later reported, which involved the preparation of 1-chloro-1-(ethylthio) derivatives of D-glucose and D-galactose from the corresponding dithioacetal pentaacetates (Scheme 6).[117] The *S,O* acetals formed were found to undergo regio- and stereoselective cyclization following deacetylation and treatment with a mixture of HgO and $HgCl_2$ in either methanol or ethanol to give the corresponding β-D-furanosides.



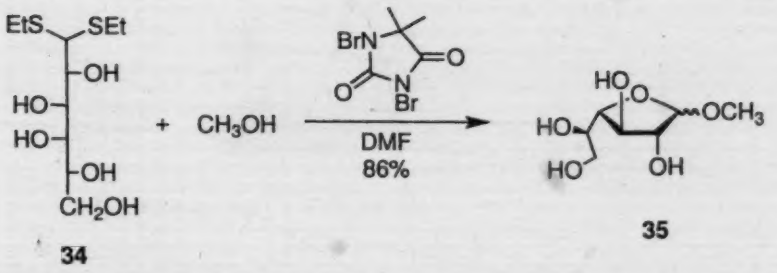
Scheme 6. Cyclization of *S,O* acetals to give ethyl β-D-galactofuranoside.[117]

An elegant extension of the synthesis of galactofuranose derivatives via the cyclization of open-chain *S,O*-acetals was later demonstrated by Hindsgaul and McAuliffe (Scheme 7).[104] Treatment of the peracetylated diethyl dithioacetal of D-galactose with acetyl chloride and boron trifluoride diethyl etherate afforded the acyclic 1-chloro-(ethylthio) derivative **27**. Glycosidation of acceptor **30** with donor **27** in the presence of AgOTf and Ag_2CO_3, provided *S,O*-acetal **31**. Deacetylation of **31** under Zémplen conditions,[118] followed by addition of HgO and $HgCl_2$ to the methanolic solution and subsequent acetylation gave β-galactofuranoside **33**.



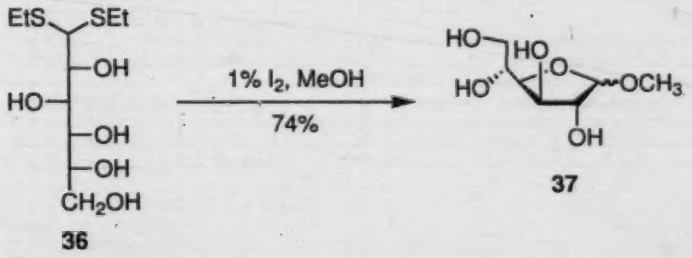
Scheme 7. Synthesis of galactofuranosides via cyclization of open-chain *S,O*-acetals.[104]

Relevant to these methods is a recent report involving the 1,3-dibromo-5,5-dimethylhydantoin (DBDH)-mediated activation of glycosyl dithioacetals leading to the formation of glycofuranosides (Scheme 8).[119]



Scheme 8. DBDH-mediated dithioacetal cyclization with methanol.[119]

A major disadvantage of these methods, however, is the use of toxic heavy metal salts and expensive reagents. A related methodology that circumvents this limitation is the treatment of glucosyl, mannosyl and arabinosyl dithioacetals with a dilute solution of iodine in methanol to give the corresponding glycosyl furanosides.[120] As shown in Scheme 9, stirring dithioacetal **36** in 1% I_2 in methanol for 32 hours afforded glucofuranoside **37** in 74% yield.



Scheme 9. Treatment of D-glucose diethyl dithioacetal with iodine and methanol.[120]

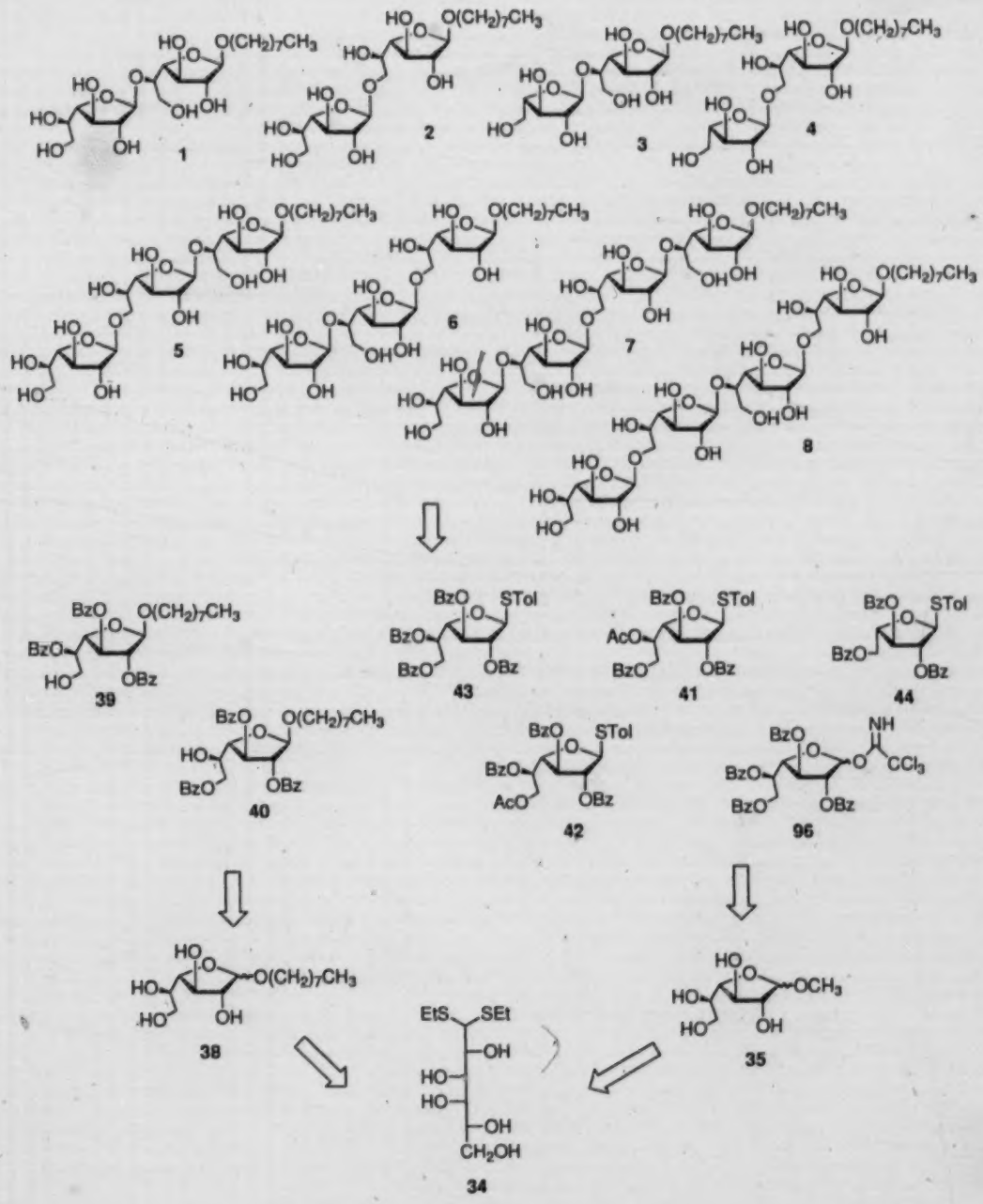
This reaction was only applied to arabinose, glucose and mannose-derived dithioacetals, but not to those derived from galactose. To date, this is the only reported use of iodine in methanol to cleave dithioacetals leading to the formation of alkyl furanosides. However, the use of iodine in methanol for the activation of

thioglycosides,[121] cleavage of TBDMS ethers,[122] cleavage of trityl and dimethoxytrityl ethers,[123] and cleavage of tetrahydropyranyl ethers[124] has been reported.

2.2 Synthetic Strategy.

The strategy designed to prepare target compounds **1–8** started with the use of dithioacetal **34** as the precursor of the galactofuranose ring as shown in Scheme 10. The rationale for using this approach is discussed in detail below. We envisioned that the synthesis of octyl galactofuranosides **38** and methyl galactofuranoside **35** could be achieved through cyclization of **34** in the presence of iodine and either methanol or *n*-octanol. Methyl glycoside **35** could serve as the precursor to thioglycoside donors **41–44**, as well as imidate donor **96**. On the other hand, octyl glycoside **38** would lead to **39** and **40**, which were used as the precursor to the reducing end of the molecules. The use of octyl aglycon in these substrates was expected to facilitate[125] the enzyme assays performed during kinetic studies of GlfT2. In addition, it has previously been demonstrated that oligosaccharides with long chain aglycons are better substrates for mycobacterial glycosyltransferases than glycosides of short chain alcohols.[87]

Thioglycosides were chosen as glycosyl donors for most of the glycosylations as they are easy to prepare, easily stored due to their stability, readily activated for glycosylation and can be straightforwardly converted to other glycosyl donors should the need arise.[126] A variety of reagents such as bromine followed by silver triflate or mercuric cyanide,[127] methyl triflate,[128] dimethyl(methylthio)sulfonium triflate (DMTST),[129] iodonium dicollidine perchlorate (IDCP),[130] N-iodosuccinimide (NIS)–TfOH,[131,132] NIS–silver triflate (AgOTf),[131] iodine,[121] 1-benzenesulfinyl piperidine (BSP)



Scheme 10. Monosaccharide building blocks.

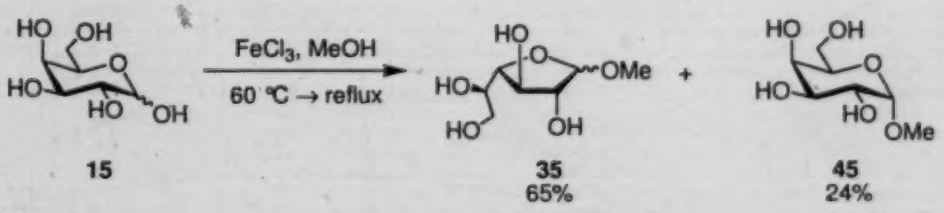
–triflic anhydride,[133] and diphenyl sulfoxide–triflic anhydride[134] can be used to activate thioglycosides. Among these promoters, NIS–TfOH[131] is perhaps the most widely used but we chose to use NIS–AgOTf[132] as the promoter system in our glycosylation reactions as silver triflate is an easily measured solid while triflic acid is a highly-corrosive liquid, which is more difficult to manipulate, particularly in small-scale reactions.

The reactivity of thioglycosides using the NIS–AgOTf promoter system can best be explained through the hard-soft-acid-base concept. Both sulfur and iodine are large and highly polarizable soft ions. Sulfur acts as a soft nucleophile readily accepting a positive charge and is thus activated once it attacks the equally soft electrophile iodonium ion produced *in situ* from NIS and AgOTf. The resulting sulfonium ion then acts as a leaving group that, upon departure, forms an oxacarbenium intermediate. Attack of a nucleophile (the alcohol) on the oxacarbenium affords the desired glycoside.

In some cases, it was advantageous to use trichloroacetimidate **96** as the donor for the coupling reactions. This compound could be easily prepared from thioglycoside **43**. With regard to protecting group selection, benzoyl esters were used in both the donors and acceptors to facilitate one-step deprotection in the final step. Moreover, the presence of participating benzoyl group C-2 in the donor would lead to the desired 1,2-*trans*-β-galactofuranosides with high diastereoselectivity in the glycosylation reactions. In some cases, acetyl groups were also incorporated into the thioglycoside donors (**41** and **42**). Donors with these two different types of acyl groups allowed the addition of other sugar residues by simply performing a selective deacetylation, thus yielding an alcohol ready for glycosylation.

2.3 Preparation of Thioglycoside Donors.

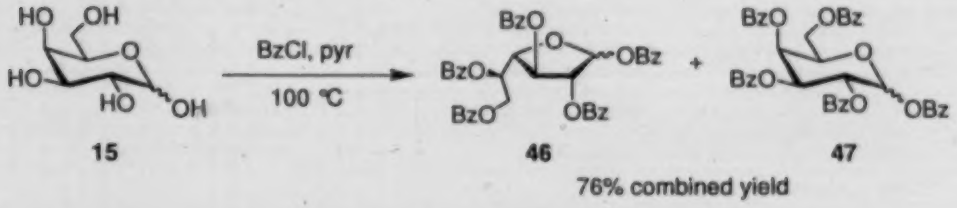
The synthetic strategy used for the preparation of the thioglycoside donors involved the use of methyl galactofuranoside intermediate **35**. During the initial stages of this project, a number of strategies were explored for the synthesis of this methyl glycoside, before the general approach outlined in Scheme 10 was chosen. We first tried to access **35** via a kinetically controlled Fischer glycosylation of D-galactose using methanol in the presence of $FeCl_3$ as previously described (Scheme 11).[99] However, mixtures of methyl pyranosides (**45**) and methyl furanosides (**35**) were obtained, which were very difficult to separate chromatographically, especially when done on large scale. Although some of the modifications of this reaction could have been explored (e.g., use of calcium chloride as an additive[101]), in all cases mixtures of furanosides and pyranosides are formed and thus this separation problem would remain. Therefore, we abandoned this approach.



Scheme 11. Preparation of methyl D-galactofuranoside **35** via Fischer glycosylation.

The previously reported high temperature benzoylation of galactose[102] was also explored (Scheme 12). This, however, proved unsuccessful, as obtaining a pure crystalline form of the expected peracetylated galactofuranoside product (**46**) was difficult. Despite repeated attempts, to crystallize **46** selectively from the reaction

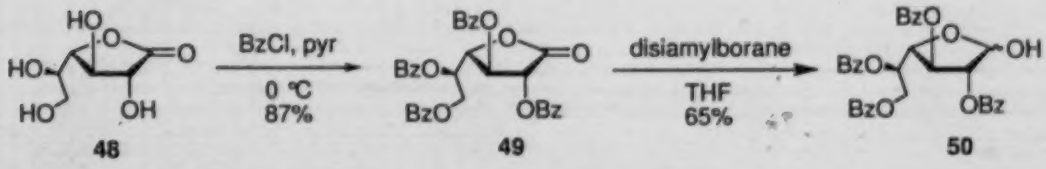
mixture as reported, the product was always contaminated with the perbenzoylated galactopyranose derivative **47**. Therefore, we also decided to not follow this approach.



15 46 47

Scheme 12. Attempted high temperature benzoylation of D-galactose.

We next studied a method that did not start from D-galactose, but instead from commercially available D-galactonolactone (**48**, Scheme 13).[135] Benzoylation of **48** using standard conditions afforded the perbenzoylated intermediate **49** in 87% yield. Reduction of intermediate **49** with disiamylborane afforded **50** in 65% yield. Although reasonable yields were obtained using this synthetic route, it was deemed impractical due mainly to the expensive starting material (**48**) as well as expensive and toxic reagents required.



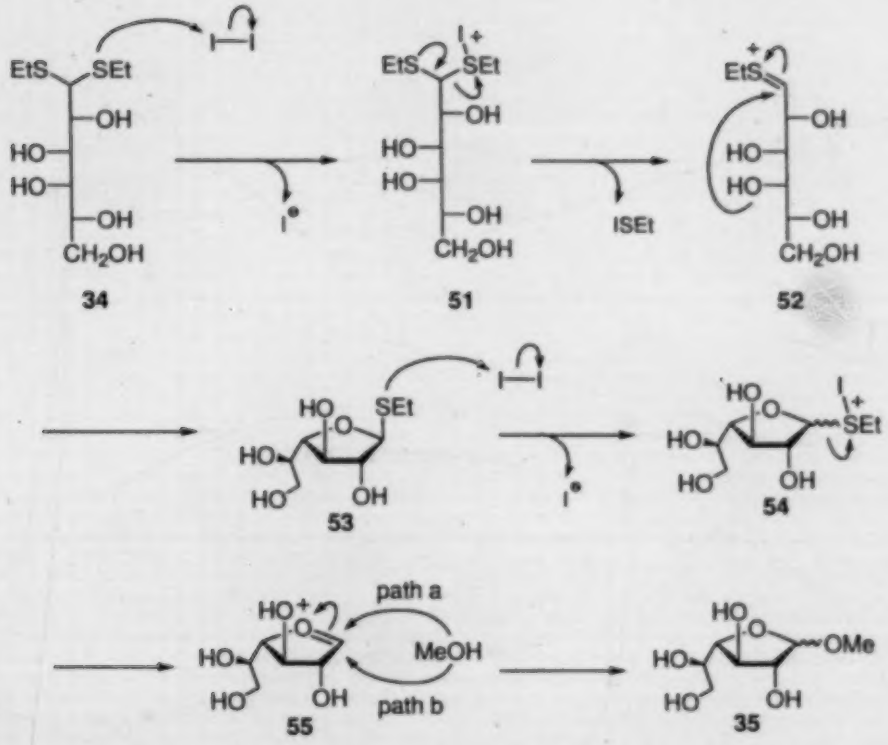
48 49 50

Scheme 13. Galactofuranose derivatives via reduction of benzoylated galactonolactone.

Faced with these problems, we then decided to employ the previously reported dithioacetal cyclization approach. Rather than use toxic mercury salts to promote this reaction, we chose instead to use iodine.[120] As mentioned earlier, this reaction had not

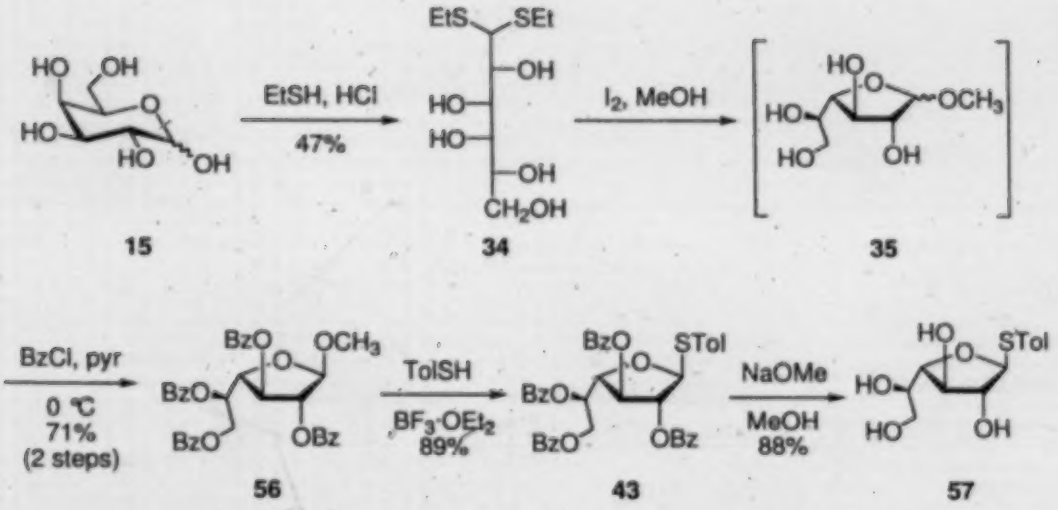
previously been applied to dithioacetals derived from D-galactose and thus this represents the first application of the method to the synthesis of galactofuranosides. The major advantage of this reaction is its simplicity. Not only does this technique avoid the use of toxic heavy metals, it is also inexpensive, convenient, versatile and, most importantly, provides high yield of galactofuranosides without the formation of galactopyranosides. This prompted us to apply this methodology in preparing methyl galactofuranoside.

The proposed mechanism of the cyclization reaction is shown in Scheme 14. As mentioned earlier, one of the sulfur atoms of **34** attacks the electrophilic iodine to form sulfonium ion **51**. The sulfonium ion, being a good leaving group, can then be easily cleaved to form thiocarbenium **52**. Subsequent cyclization of **52** gives thioglycoside **53**, which then reacts with another molecule of iodine to generate **54**. Loss of ethyl sulfenium iodide leads to oxacarbenium intermediate **55**, which can be attacked by methanol thus leading to methyl glycoside **35**.



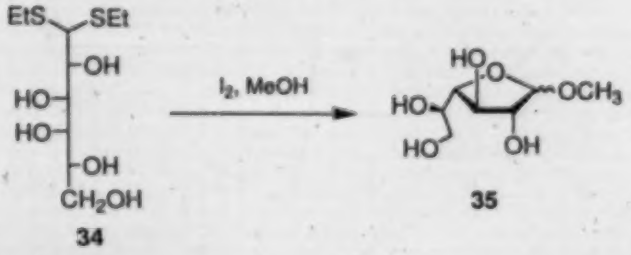
Scheme 14. Proposed mechanism of iodine-induced dithioacetal cyclization in methanol.

To implement this approach, D-galactose was treated with ethanethiol and HCl to give diethyl dithioacetal **34** in 47% yield (Scheme 15).[136] Table 1 shows the different iodine concentrations used to obtain the optimal conditions in preparing methyl galactofuranoside **35**. Although higher concentrations of iodine (3–5%) resulted in shorter reaction times, we opted to use the 2% iodine concentration. This was due to the formation of large amounts of salts at higher iodine concentrations; these salts interfered in the subsequent benzoylation step (below). Thus, cyclization of **34** was achieved upon treatment with 2% I_2 (by weight, 0.08 M) in methanol to give **35** (Scheme 15). The reaction was quenched by adding solid sodium thiosulfate until the solution



Scheme 15. Preparation of thioglycoside **57** via cyclization of dithioacetal **34**.

Table 1. Synthesis of **35** as a function of iodine concentration.



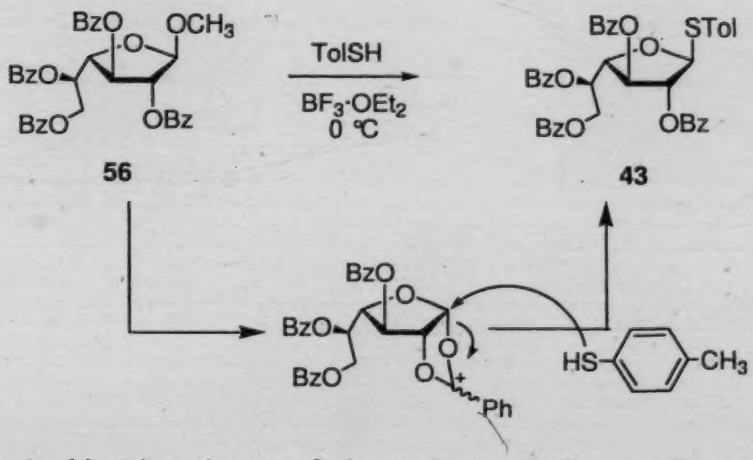
Concentration (w/v)	Time (h)
1%	24
2%	8
3%	3
4%	2
5%	<1

turned colorless. The solution was then neutralized with solid sodium bicarbonate and evaporation of the solvent *in vacuo*. The formation of salts was also observed during

work-up and the polarity of the product made purification difficult. Therefore, pyridine was added to the crude methyl galactofuranoside product, followed by the dropwise addition of benzoyl chloride. The resulting product, **56**, was obtained in 71% yield after purification by chromatography over two steps.

The β-configuration in **56** was established using NMR spectroscopy. The singlet signal of the anomeric hydrogen (δ_H = 5.80 ppm) establishes the *trans* relationship between H-1 and H-2. A *cis* relationship between H-1 and H-2 would have shown a larger coupling constant ($J_{1,2}$ = 3–5 Hz).[137] Also, the chemical shift of the anomeric carbon of **56** (δ_c = 106.9 ppm) is downfield compared to the more upfield shift (δ_c ~ 103 ppm) of the anomeric carbon of an α-product.[137] In addition to this, the C-2 and C-4 signals resonated at 82.2 and 81.2 ppm, respectively. These signals are significantly more downfield than these carbons in pyranosides and thus establish the furanose ring form in **56**. The observed ^{1}H NMR and ^{13}C NMR data is consistent with the previously reported characteristic signal pattern for β-D-galactofuranosides.[137]

Compound **56** was then converted to the perbenzoylated thioglycoside **43** in 89% yield upon treatment with boron trifluoride diethyl etherate ($BF_3{\cdot}OEt_2$) and *p*-thiocresol. The observed β-stereoselectivity of the reaction was achieved via the anchimeric assistance of the benzoyl group at the C-2 position as illustrated in Scheme 16. Reaction of methyl glycoside **56** with the Lewis acid leads to oxacarbenium ion that, through neighboring group participation of the benzoyl group, leads to dioxolenium ion **58**. The formation of **58** thus results in the *p*-thiocresol attacking from the β-face to give the desired product, **43**.



Scheme 16. Anchimeric assistance of a benzoyl group leading to 1,2-*trans*-selectivity.

The assigned β-configuration was confirmed upon analysis of its ^{1}H NMR spectrum of **43**, in which the anomeric hydrogen appeared as a doublet with $J_{1,2}$ = 2.0 Hz. This value is indicative of a *trans* relationship between H-1 and H-2; for thioglycosides in which these two hydrogens are *cis*, a $J_{1,2}$ = 4–6 Hz would have been expected. Furthermore, the drastic upfield shift of C-1 (δ_c = 91.6 ppm) in the ^{13}C NMR spectrum of the product lends credence to the proposed structure of the product (i.e., the presence of sulfur at C-1). The upfield shift observed for the resonance for the anomeric carbon of thioglycosides, when compared to that of *O*-glycosides (δ_c ~ 105 ppm), is attributed to the lower electronegativity of the sulfur compared to oxygen.

The tetrabenzoylated thioglycoside **43** was then debenzoylated under Zémplen conditions using sodium methoxide in methanol to give compound **57** in 88% yield (Scheme 15). Upon inspection of the ^{1}H NMR spectrum of the deprotected product, the anomeric hydrogen signal showed an unexpected coupling constant value ($J_{1,2}$ = 5.0 Hz). This value suggests an α-conformation, but we viewed the epimerization of the anomeric

centre upon deprotection as very unlikely. This deviation from what we was expected for the deprotected β-thioglycoside ($J_{1,2}$ ~ 2.0 Hz) is in agreement with a previously reported observation.[138] In this earlier study, the authors observed that the benzoylated thioglycosides showed similar vicinal coupling constant to the corresponding *O*-glycosides. Although these rings are expected to be flexible, and that the experimental coupling constants will be an average of those obtained for all conformers, the small $J_{1,2}$ in the protected β-galactofuranosides suggests the substituent at the anomeric position spends most of its time in a quasi-axial position, which is favored by the anomeric effect.[138] This is observed when the ring is in the ^{1}E, $^{1}T_{O}$ or E_{O} conformation (Figures 18 and 19). In addition, these conformers place the large benzoyl groups at C-2 and C-3 far

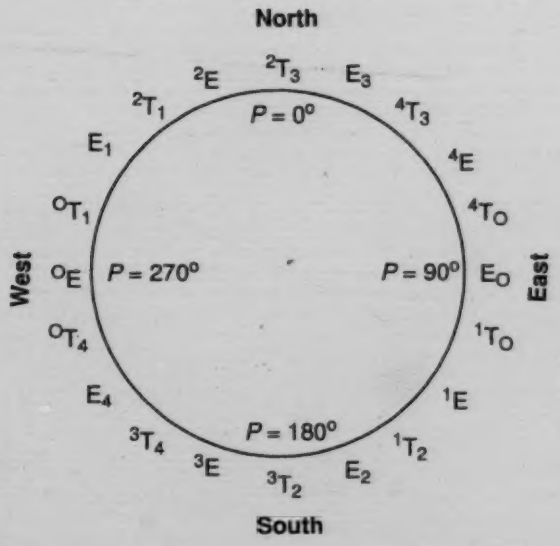


Figure 18. Pseudorotational itinerary for a D-galactofuranose ring.

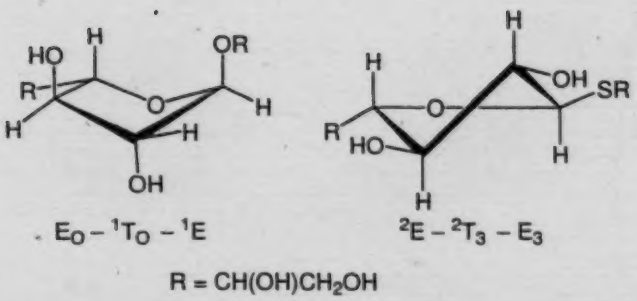
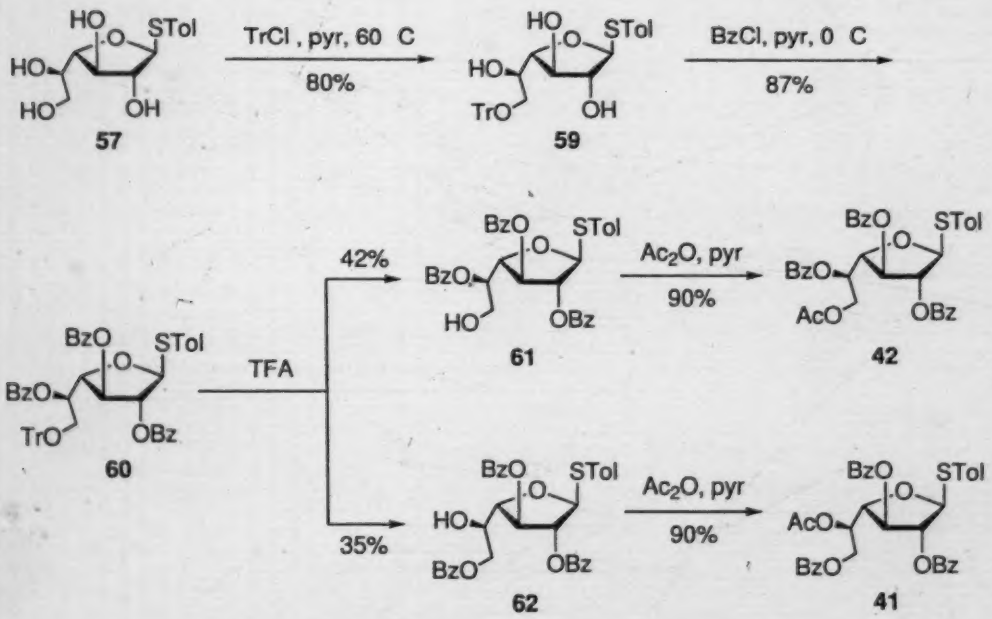


Figure 19. Conformations of 1-*O* and 1-*S*-galactofuranosides based on $J_{1,2}$.

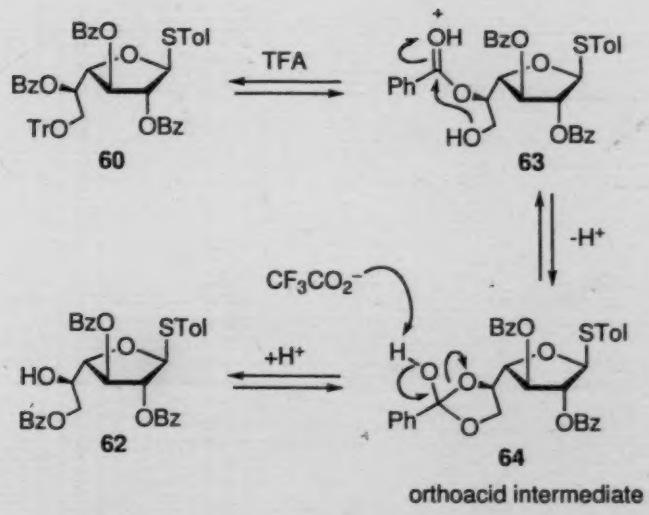
away from each other. Once deprotected, however, the thioglycosides derivatives undergo a conformational shift towards the ^3E–$^2T_3$–E_3 region of the pseudorotational wheel, where the anomeric sulfur atom substituent is in a quasi-equatorial position (Figure19).[138] This change could be attributed to a weaker anomeric effect of sulfur compared to the oxygen[139] and reduced negative steric interactions between the groups on C-2 and C-3.

With the deprotected thioglycoside **57** in hand and its structure confirmed, the 6-hydroxyl group was then tritylated under standard conditions followed by the protection of the three free hydroxyl groups with benzoyl groups (Scheme 17). The exclusive protection of the primary hydroxyl group was ensured by the use of a bulky trityl group. The fully protected compound **60** was obtained in 70% yield over the two steps. The trityl group was then cleaved using trifluoroacetic acid in wet dichloromethane to afford **61** and **62** in 33% and 44% yield, respectively.



Scheme 17. Synthesis of donors **41** and **42**.

Compound **62** was formed via acid-catalyzed acyl migration of the benzoyl group from O-5 to O-6 (Scheme 18). The migration of the acyl group could occur through nucleophilic attack of the C-6 hydroxyl group in **63** on the adjacent protonated benzoate ester to form orthoacid intermediate **64**. Subsequent deprotonation by a trifluoroacetate anion collapses the orthoacid intermediate to give compound **62**. Although normally this migration would be viewed as undesirable, it is of advantage to us because it leads to the two desired compounds in a single step. It is important to emphasize that both intermediates **61** and **62** are valuable intermediates in our synthetic route. The structures of **61** and **62** could be readily differentiated by NMR spectroscopy of the products. In **61**, the signal for the H-5 resonance is significantly downfield (5.69 ppm) as would be expected as O-5 is acylated.

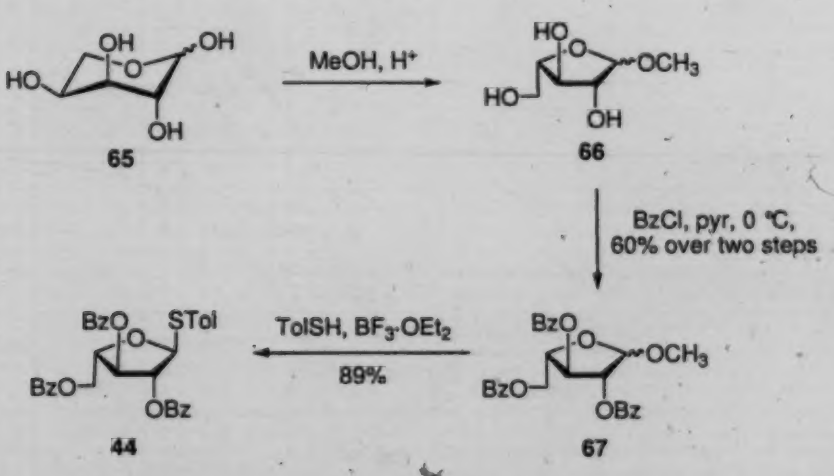


Scheme 18. Acyl migration of benzoyl group from O-5 to O-6 under acidic conditions.

In contrast, in the NMR spectrum for **62**, H-5 appears at 4.59 ppm. Subsequent acetylation of **61** and **62** with acetic anhydride in pyridine provided donors **41** and **42** in excellent yields (Scheme 17). The structures of **41** and **42** were confirmed upon inspection of their ^{1}H NMR spectra, which showed the presence of the acetate methyl group around 2.00 ppm. In addition, compared to the parent alcohols **61** and **62**, acetylation resulted in a downfield shift of H-6_a/H-6_b and H-5, respectively. Acetylation of **61** and **62** not only confirmed the structural assignments for the products arising from the acyl migration step, but also provided intermediates **41** and **42**, which are excellent precursors for chain extension after selective deprotection of the acetyl group.

The synthesis of thioglycoside donor **44**, which was to be used for the preparation of the L-arabinose-containing targets, is shown in Scheme 19. Commercially available L-arabinose (**65**) was treated with acetyl chloride in methanol to afford intermediate **66**.[140] Without further purification, intermediate **66** was benzoylated under standard conditions

to give **67** in 60% yield overall yield. This was followed by treatment of **67** with *p*-thiocresol in the presence of boron trifluoride diethyl etherate to obtain donor **44** in 89% yield. Trace amounts of the α-anomer were also produced, but were easily separated during chromatography. Analysis of the ^{13}C NMR spectrum showed an upfield shift of C-1 to δ_c = 91.6 ppm, which confirmed that the thioglycosylation had indeed occurred. The ^{1}H NMR spectrum also showed H-1 as a singlet at 5.77 ppm, corresponding to the β-configuration.[141]

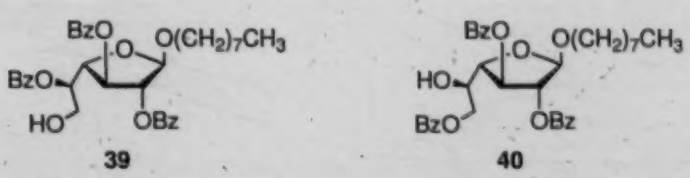


Scheme 19. Synthesis of thioglycoside donor **44**.

2.4 Preparation of Octyl Glycoside Acceptors.

As mentioned above, we chose to synthesize these glycans as octyl glycosides based on previous studies demonstrating that oligosaccharides containing long chain aglycons are better substrates for mycobacterial glycosyltransferases than glycosides of short chain alcohols.[87] The octyl group in the aglycon would also help to facilitate the purification of the products of the enzyme assay during GlfT2 kinetic studies.[125]

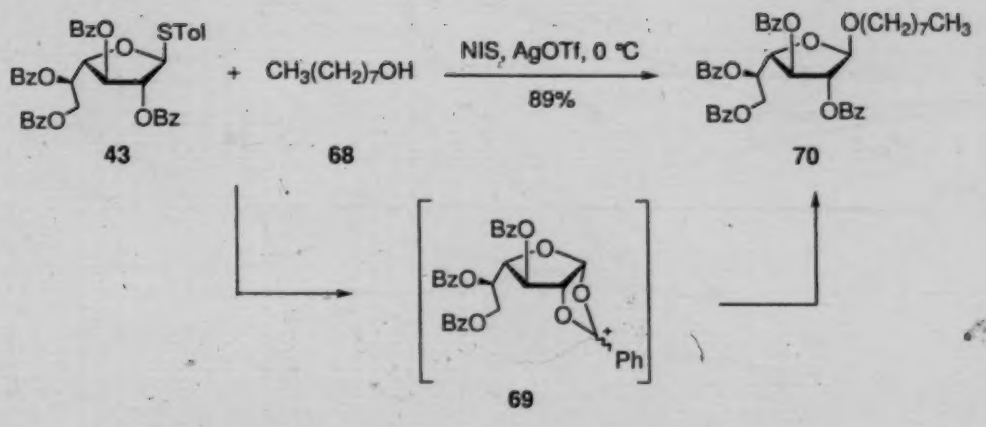
Furthermore, the octyl group has been used in substrates for studies of mycobacterial arabinosyltransferases and other glycosyltransferases.[142,143] Octyl glycosides **39** and **40** (Scheme 20) were chosen as building blocks for the reducing end of the target compounds.



Scheme 20. Structures of octyl glycoside acceptors **39** and **40**.

2.5 Synthesis of Octyl Galactofuranosides Acceptors.

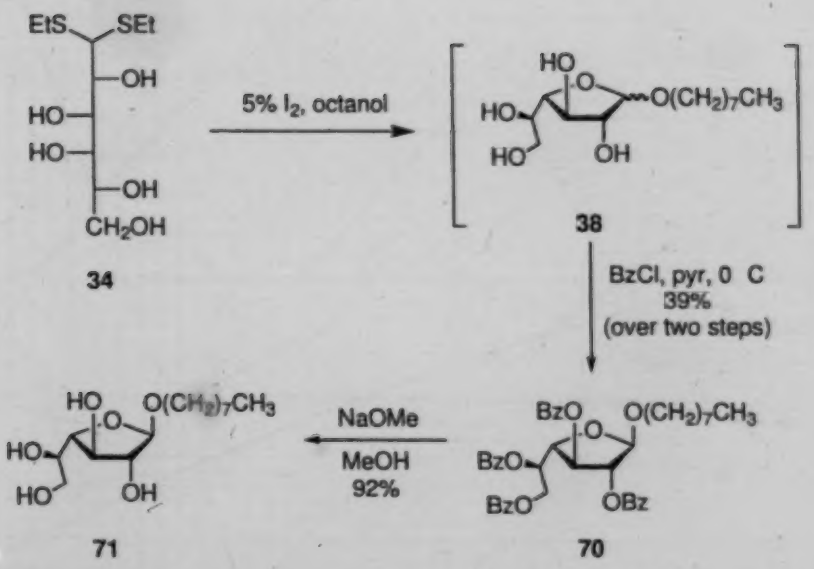
During the initial stages of this project, the fully benzoylated octyl glycoside **70** was prepared from thioglycoside **43** (Scheme 21). This was accomplished by coupling thioglycoside donor **43** with *n*-octanol (**68**) upon activation with NIS and AgOTf, leading to the exclusive formation of the β-anomer in 89% yield. This result was not unexpected due to the anchimeric assistance of the benzoyl group at the C-2 position. Other reported methods that we could have used include a Lewis acid-catalyzed ($SnCl_4$) coupling reaction of 2,3,5,6-tetra-*O*-acetyl-β-galactofuranose and *n*-octanol that gave a fully acetylated octyl galactofuranoside in 72% yield.[142,143]



Scheme 21. Synthesis of octyl glycoside **70** from donor **43**.

Although this approach was successful, we wanted to prepare **70** from D-galactose in fewer steps. Fischer glycosylation of D-galactose with *n*-octanol is a possibility and this has been reported;[144] however, this method suffers from poor product diastereoselectivity - an α:β ratio of 1:4–5 is formed, depending upon conditions. Inspired by the successful preparation of methyl galactofuranoside via cyclization of galactose diethyl dithioacetal in the presence of iodine and methanol, we prepared octyl glycoside **71** using the same approach (Scheme 22). The use of this strategy allowed us to reduce the number of synthetic steps thereby making the overall synthesis more efficient.

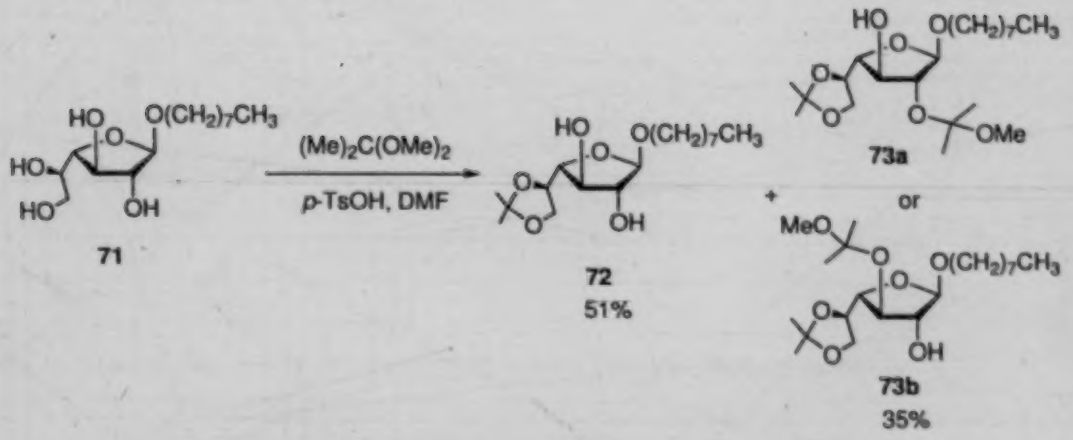
Thus, to dithioacetal **34**, 5% iodine in *n*-octanol (by weight) was added and the mixture was stirred for 24 hours. Next, the crude product obtained after workup and removal of the *n*-octanol was suspended in pyridine before benzoyl chloride was added. While benzoylation was not essential, doing so facilitated isolation of the product, **70**, which was obtained as a 9:1 β:α mixture.



Scheme 22. Synthesis of octyl glycoside via dithioacetal cyclization.

The β-glycoside was successfully purified by chromatography and subsequent treatment with sodium methoxide thus afforded **71** in 36% yield in three steps from **19**. The formation of the β-anomer as the major product was attributed to steric factors. The anomeric hydrogen of the product appeared as a singlet at 5.24 ppm while the ^{13}C NMR spectrum showed the anomeric carbon at 105.7 ppm, which clearly establishes the assigned β-configuration.[137]

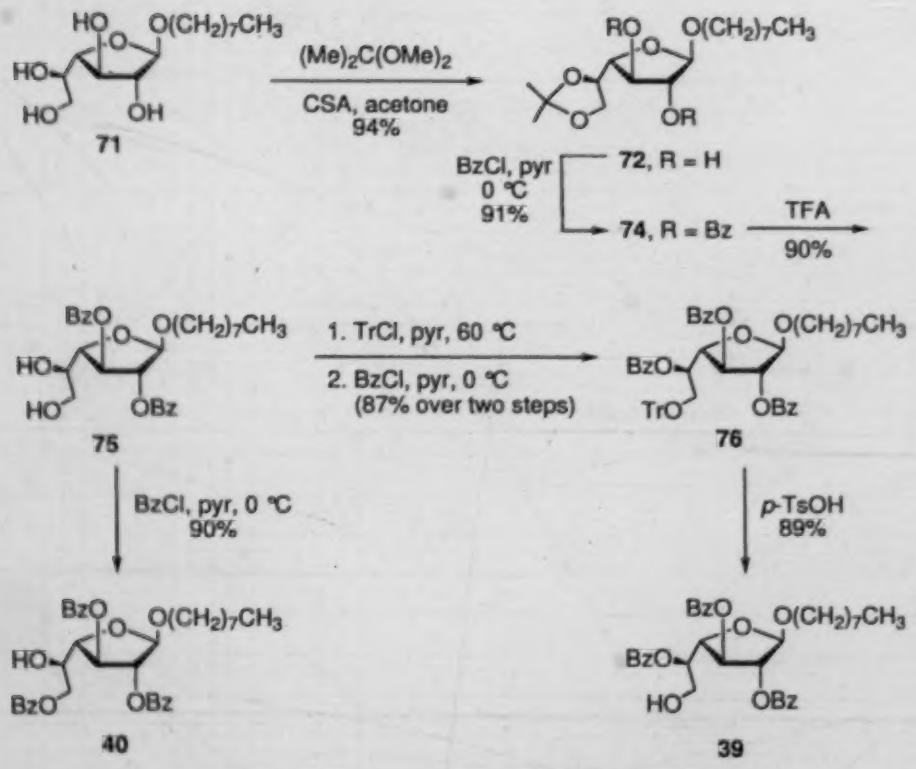
With two possible routes for the synthesis of **71** in place, the hydroxyl groups at C-5 and C-6 were then protected by reaction with dimethoxypropane in DMF using *p*-TsOH to afford isopropylidene ketal **72** in 51% yield (Scheme 23). The observed low yield was attributed to the formation of side product **73** (which can either be **73a** or **73b**) in 35% yield containing two acetals, one cyclic and one acyclic. This problem was



Scheme 23. Isopropylidene protection of octyl glycoside **72**.

circumvented by reducing the amount of dimethoxypropane (2 eq) and using dry acetone as the solvent. Under these conditions, **72** was obtained in 94% yield (Scheme 24). The installation of the ketal protecting group was characterized through the appearance of two singlets at 1.62 and 1.46 ppm, corresponding to the isopropylidene methyl groups, in the ^{1}H NMR spectrum and the appearance of a signal due to a quaternary C (ketal group) at 109.9 ppm in the ^{13}C NMR spectrum.

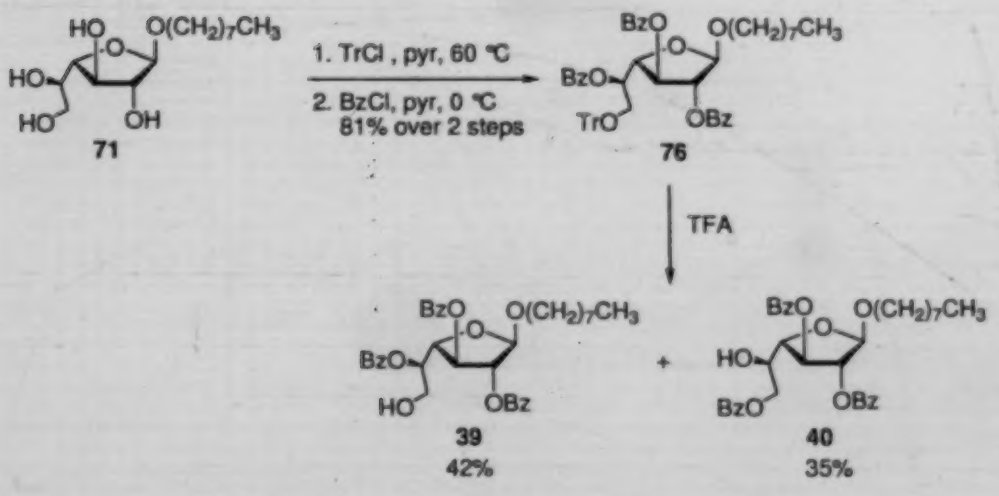
The final steps in the synthesis of octyl glycosides **39** and **40** are outlined in Scheme 24. Benzoyl protection of diol **72** using benzoyl chloride in pyridine to gave intermediate **74** in 91% yield. The ^{1}H NMR spectrum of **74** showed the presence of aromatic hydrogens as multiplet at 7.61–7.21 ppm while the ^{13}C NMR spectrum showed the chemical shifts of the two C=O groups at 165.6 and 165.3 ppm. As an expected consequence of benzoylation, a downfield shift of H-2 and H-3, to 5.42 and 5.44 ppm, respectively, was also observed. The isopropylidene protecting group was then cleaved with trifluoroacetic acid as previously described[143] to produce diol **75** in 90% yield.



Scheme 24. Synthesis of octyl glycosides **39** and **40**.

The C-6 hydroxyl group in **75** was then tritylated upon treatment with trityl chloride in pyridine at 60 °C. Without purification of the tritylated intermediate, the remaining hydroxyl group was benzoylated to furnish **76** in 87% yield over two steps. Cleavage of the trityl group in **76** using *p*-TsOH in methanol–dichloromethane gave octyl glycoside **39** in 89% yield. To prepare octyl glycoside **40**, diol **75** was treated with a limiting amount of benzoyl chloride (1.1 equivalents) in pyridine. The structure of the product (**40**) was confirmed using ^{1}H NMR, which showed a downfield shift of H-6_a and H-6_b due to the expected effect of the benzoyl group attached to O-6.

It was also possible to prepare **39** and **40** using an approach analogous to the synthesis of thioglycosides **61** and **62**, which involved an acid-catalyzed acid migration step. Thus (Scheme 25), treatment of compound **76**, prepared from intermediate **71**, with trifluoroacetic acid in wet dichloromethane gave octyl glycosides **39** and **40** in 42% and 35% yield, respectively. The two compounds were easily identified upon evaluation of their ^{1}H NMR spectra. The resonance for H-5 in **39** appeared more downfield (5.64 ppm) due to the presence of the benzoyl group at O-5 while the H-5 resonance of **40** was present at a more upfield frequency, 4.50 ppm. This approach has also been used by Pathak and co-workers[143] to prepare selectively protected galactofuranoside acceptors.



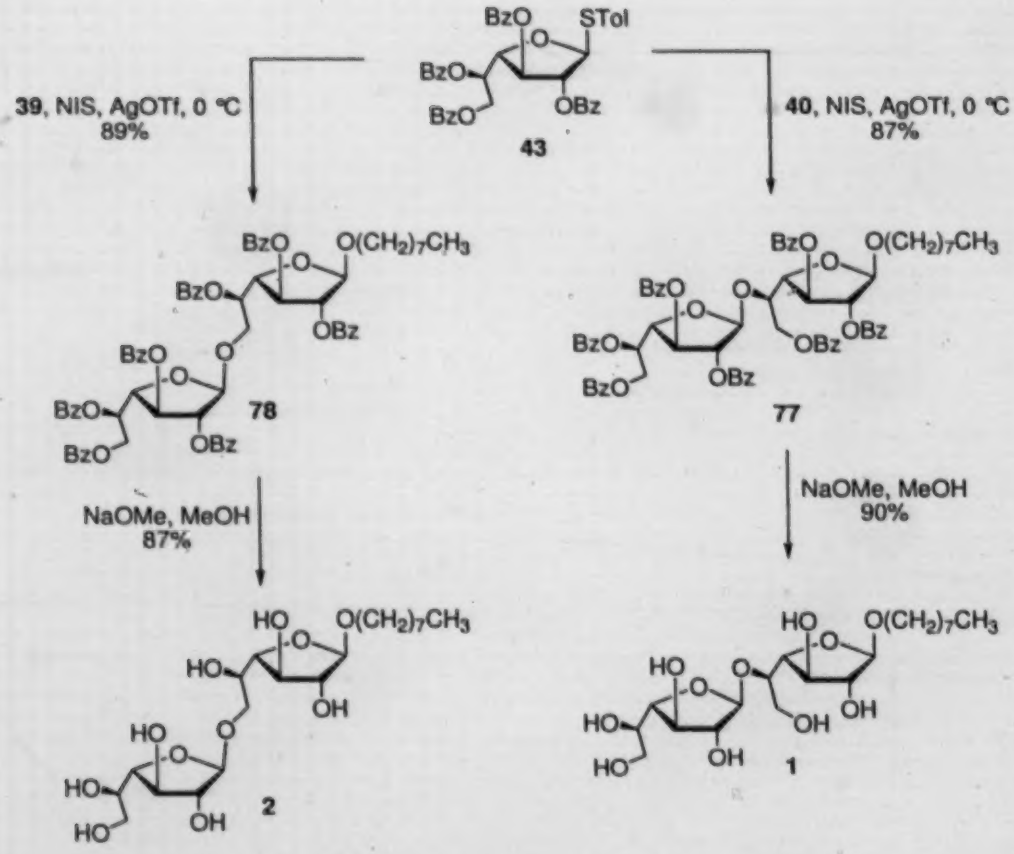
Scheme 25. Preparation of octyl glycosides **39** and **40** using TFA.

2.6 Synthesis of Oligosaccharides.

2.6.1 Synthesis of Disaccharides 1–4.

In the previous sections the preparation of the monosaccharide building blocks was described and once these routes were in place, we could explore the synthesis of the oligosaccharide targets. A number of protocols for the preparation of glycosides of galactofuranose disaccharides have been reported.[101,104,145-150] These include the use of $SnCl_4$,[142,143] NIS–TESOTf,[101] NIS–$Sn(OTf)_2$[143] and TMSOTf[145] as promoters with acetate, thioglycoside and imidate donors. In our case, disaccharides **1** and **2** were prepared from the NIS–AgOTf-promoted coupling of either octyl glycoside **39** or **40** with thioglycoside donor **43,** as outlined in Scheme 26. As mentioned previously, the use of AgOTf during the glycosylation step afforded an added advantage due to its ease of handling as opposed to using TESOTf or TfOH.

The preparation of **1** began with the coupling of octyl glycoside **40** and thioglycoside **43** upon activation with NIS and a catalytic amount of AgOTf to give the perbenzoylated disaccharide **77** in 87% yield (Scheme 26). Treatment of **77** with sodium methoxide in methanol provided disaccharide **1** in 90% yield. Examination of the ^1H NMR spectrum showed two anomeric hydrogens appearing at 5.22 and 4.96 ppm as singlets, which are indicative of a product having two β-galactofuranoside residues.[137] In addition, the (1→5) linkage was confirmed by a Heteronuclear Multiple Bond Correlation (HMBC) experiment, which showed a cross-peak between H-1' and C-5. This same experiment showed a correlation between H-1 and the $O\underline{C}H_2R$ moiety of the octyl group, thus



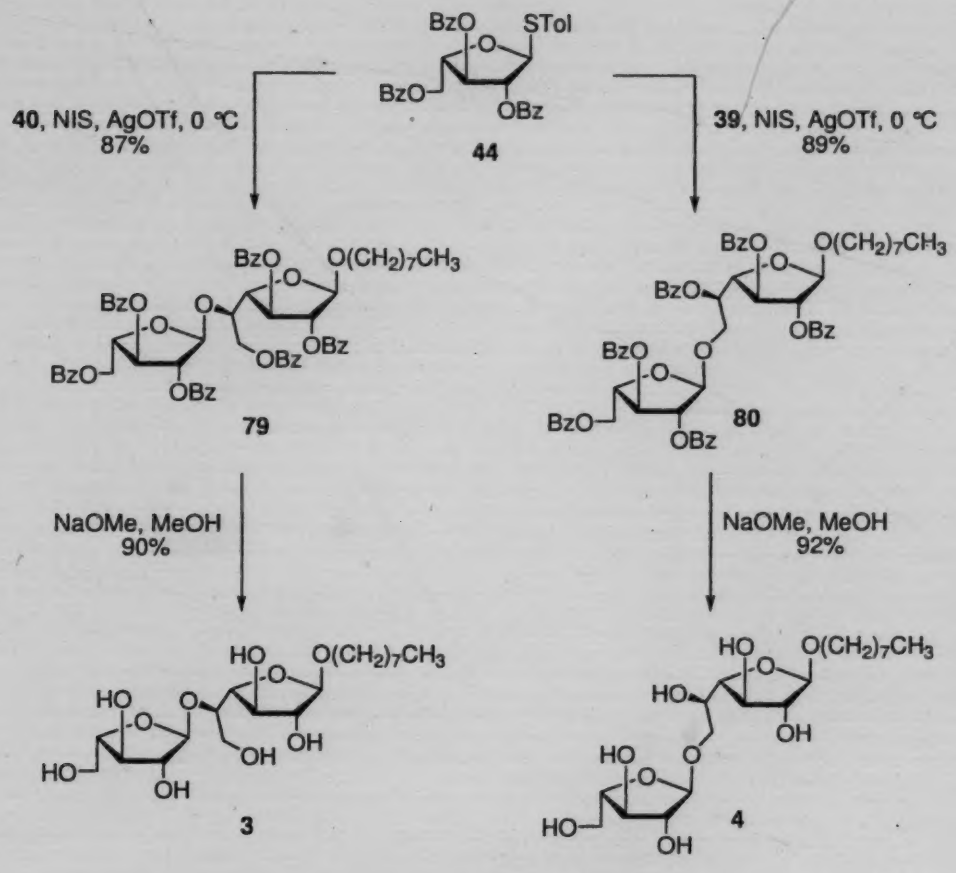
Scheme 26. Synthesis of disaccharides **1** and **2**.

confirming the linkage of the reducing end residue. The ^{13}C NMR spectrum also showed the anomeric carbons at 108.2 and 108.0 ppm, further corroborating the assigned β-configuration of disaccharide **1**.

The same synthetic approach was also used to prepare disaccharide **2** by coupling octyl glycoside **39** with thioglycoside donor **43** to obtain **78** in 89% yield (Scheme 26). Cleavage of the benzoyl protecting groups under standard Zemplén conditions gave

disaccharide **2** in 87% yield. NMR spectroscopy showed the anomeric hydrogens of disaccharide **2** at 4.91 and 4.83 ppm as doublets ($J_{1,2}$ <1.5 Hz) and the anomeric carbons were found at 108.1 and 108.0 ppm, which are the characteristic chemical shifts of a β-galactofuranoside moiety. Likewise, HMBC analysis showed correlations between H-1' and C-6 and between H-1 and the OCH_2R of the octyl group. Based on these ^1H NMR data and those obtained for other molecules synthesized here), a generalization could be made about the chemical shift of H-1 in these galactofuranosides and its correlation to linkage position. The trend observed was that H-1 involved in a (1→5) linkage resonates downfield (~ 5.2 ppm) compared to H-1 involved in a (1→6) linkage, or a linkage to an aliphatic aglycone disaccharide (~ 5.0 ppm). An evaluation of the literature demonstrates that these trends are in agreement with reported ^1H NMR chemical shifts for other galactofuranosyl disaccharides.[101,104,145-150]

Disaccharides **3** and **4** are analogs of **1** and **2**, respectively, where the non-reducing end has been replaced by L-arabinofuranosyl (Ara*f*) residue. We included **3** and **4** as target compounds for use in examining the substrate specificity of GlfT2. These compounds will be used to determine whether the presence of a terminal hydroxymethyl group is essential for GlfT2-catalyzed transfer of a Gal*f* residue from UDP-Gal*f* to the acceptor. Disaccharides **3** and **4** were synthesized using the same glycosylation approach as that of disaccharides **1** and **2** (Scheme 27).



Scheme 27. Synthesis of disaccharides **3** and **4**.

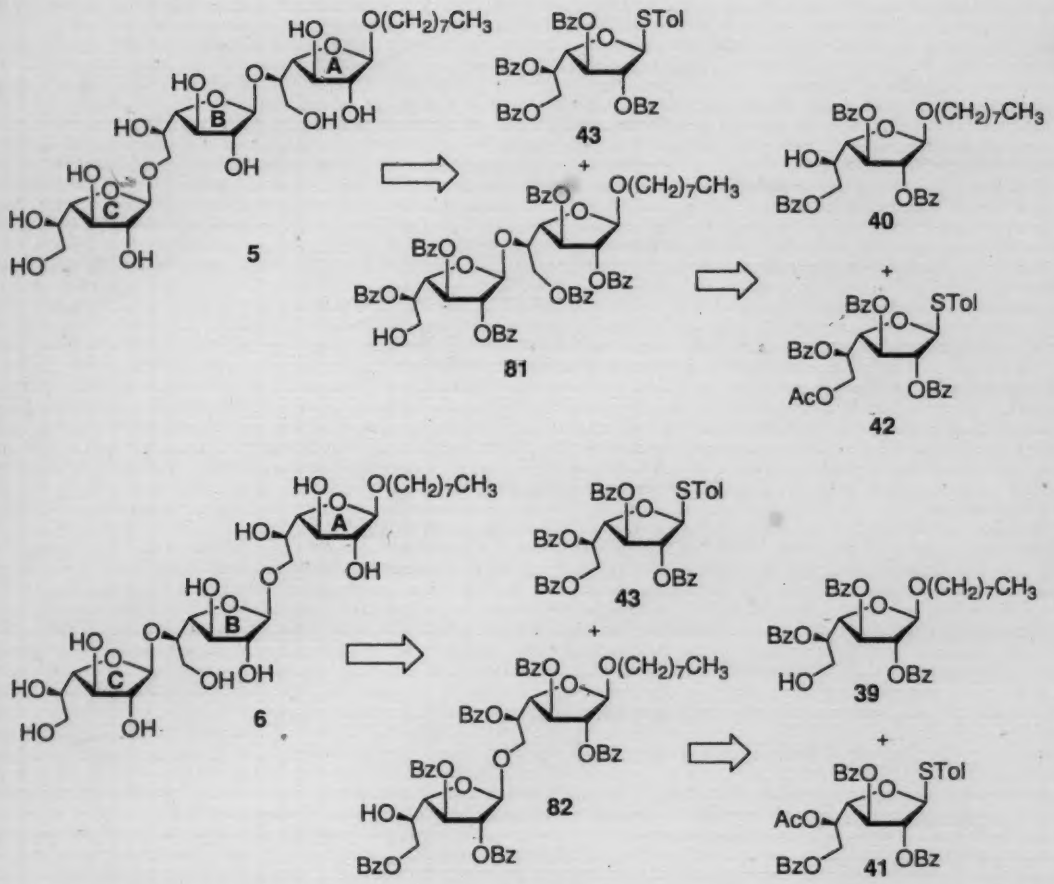
The synthesis of **3** was then initiated by coupling thioglycoside **44** with octyl glycoside acceptor **40** using NIS and AgOTf as promoters to give disaccharide **79** in 87% yield. As before, debenzoylation under standard Zemplén conditions gave disaccharide **3** in 90% yield. The ^{1}H NMR spectrum of disaccharide **3** showed the anomeric hydrogens as singlets at 5.19 and 4.82 ppm, which, again, are indicative of a product having a β-configuration and a (1→5) linkage. Further confirmation of anomeric stereochemistry was obtained from the chemical shift of the anomeric carbons, which appeared at 109.4 ppm and 109.1 ppm.

The preparation of disaccharide **4** involved first the coupling of octyl glycoside **39** and thioglycoside donor **44** to give the perbenzoylated intermediate **80** in 89% yield (Scheme 27). This was followed by debenzoylation, which afforded disaccharide **4** in 92% yield. Similar to **2**, the two anomeric hydrogens of **4** appeared at 4.91 ppm as a singlet and at 4.82 ppm ($J_{1,2}$ = 1.5 Hz) as a doublet while the anomeric carbons appeared at 110.1 ppm and 109.3 ppm. The H-1 chemical shifts observed for **3** and **4** followed the trend observed earlier for the β-(1→5)-linked and β-(1→6)-linked disaccharides **1** and **2**.

2.6.2 Synthesis of Trisaccharides 5 and 6.

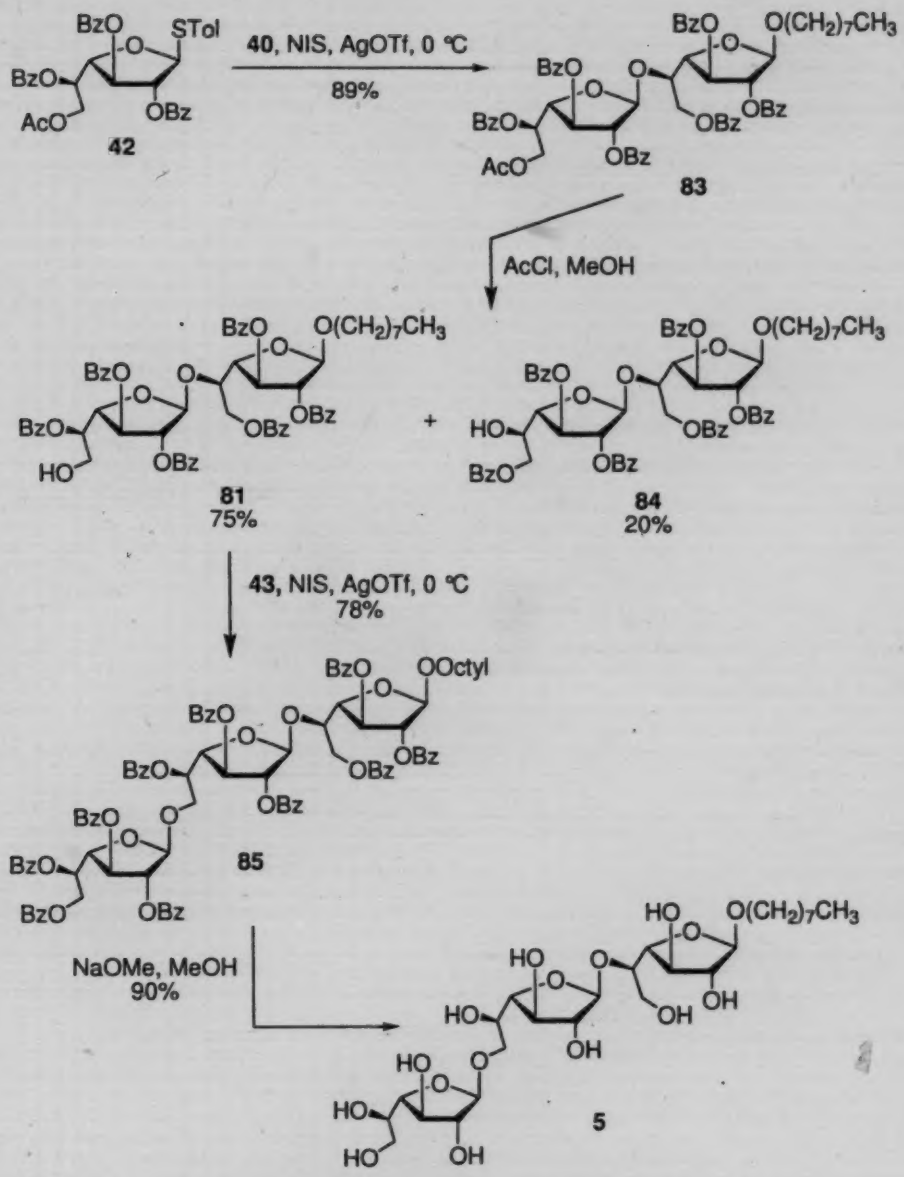
The approach used to synthesize trisaccharides **5** and **6** consisted of building the target molecule starting from the reducing end (residue A, Scheme 28) and extension of the oligosaccharide to the non-reducing end (residue C). The building blocks used in their preparation included octyl glycoside acceptors **39** and **40** and thioglycoside donors **41–43**. Unlike the synthesis of **1** and **2**, the selectively protected thioglycosides **40** and **41**, as well as fully benzoylated **43**, were used in the reactions leading to **5** and **6**.

The synthesis of **5** is detailed in Scheme 29. Octyl glycoside **40**, which contains a free hydroxyl group at C-5, was coupled with thioglycoside donor **42** upon activation with NIS and AgOTf. This reaction afforded the 6'-*O*-acetyl protected disaccharide **83** in 89% yield. The acetyl group was then selectively cleaved, in the presence of benzoyl groups, with methanolic HCl[146] to give disaccharide alcohol **81** in 75% yield. The structure of the product of the deprotection step was confirmed by the absence in the ^{1}H



Scheme 28. Retrosynthetic analysis for the preparation of trisaccharides **5** and **6**.

NMR spectrum of a singlet at ~2.0 ppm corresponding to the three hydrogens of the acetate methyl group. In addition, the pair of doublet of doublets in **83**, arising from H-6_a'and H-6_b', moved upfield, thus indicating cleavage of the acetyl group.



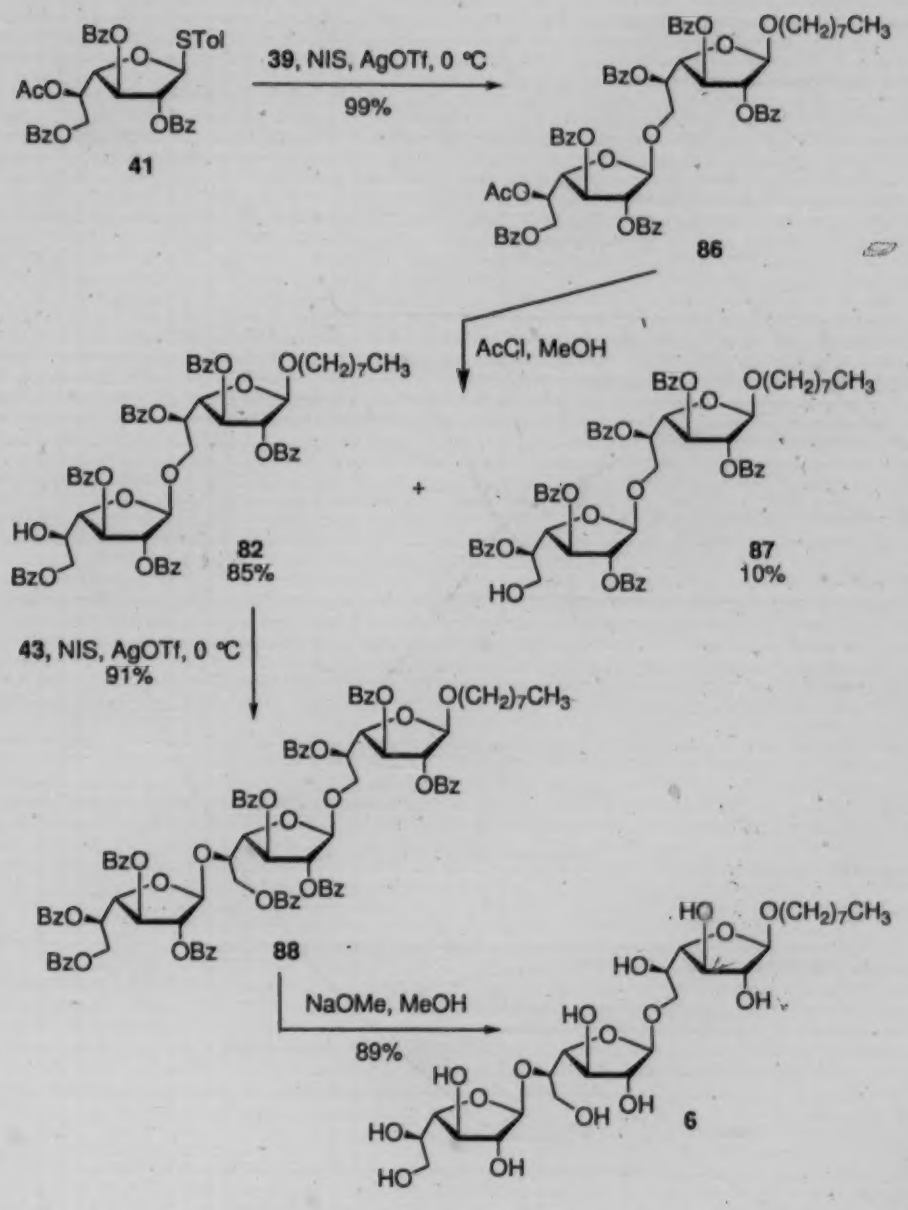
Scheme 29. Synthesis of trisaccharide **5**.

In addition to the desired product **81**, this reaction gave, in 20% yield, a by-product (**84**) arising from the migration of the benzoyl group from O-5' to O-6'. The structure of **84** was determined by ^{1}H NMR spectroscopy. The ^{1}H spectrum for **84** lacked

a signal for the acetate methyl group and showed the presence of 25 aromatic hydrogens, indicating the molecule possessed five benzoate esters. However, there was no downfield doublet or doublet of doublet resonance corresponding to H-5', indicating the absence of an acyl group at C-5'. Taken together, these data support the structure of **84**.

The synthesis of **5** was continued by the coupling of disaccharide **81** with thioglycoside **43** to give the protected trisaccharide **85** in 78% yield. This was followed by cleavage of the benzoyl protecting groups upon treatment with sodium methoxide in methanol to give trisaccharide **5** in 90% yield. The ^{1}H NMR spectrum of the product showed the anomeric hydrogens as three doublets ($J_{1,2}$ < 2.0 Hz) at 5.23, 5.04 and 4.95 ppm while in the ^{13}C NMR spectrum anomeric carbons appeared at 110.0, 109.4 and 108.9 ppm. Like the previous analyses, these NMR signals correspond to a product having a (1→6),(1→5) linkage with each residue having the β-configuration.

The same approach was also used in the synthesis of trisaccharide **6** as detailed in Scheme 30. Thus, coupling of octyl glycoside **39** with thioglycoside **41** gave the protected disaccharide **86** in 99% yield. Subsequent deprotection of the acetyl group in intermediate **86** upon reaction with HCl in methanol afforded disaccharide **82** in 85% yield. A smaller amount of the acyl migration product (**87**) was observed in this case, compared with the deprotection of **83** (see above). This can be attributed to the lesser propensity of acyl groups to migrate from primary to secondary positions.[151] The disappearance of the singlet at 1.94 ppm in the spectrum of **86**, corresponding to the acetate methyl group, and the upfield shift of H-5' from 5.88 ppm to ~4.5 ppm, confirmed that the deprotection was achieved. Disaccharide **82** was then coupled with thioglycoside **43** to give trisaccharide **88** in 72% yield; subsequent debenzoylation of **88**



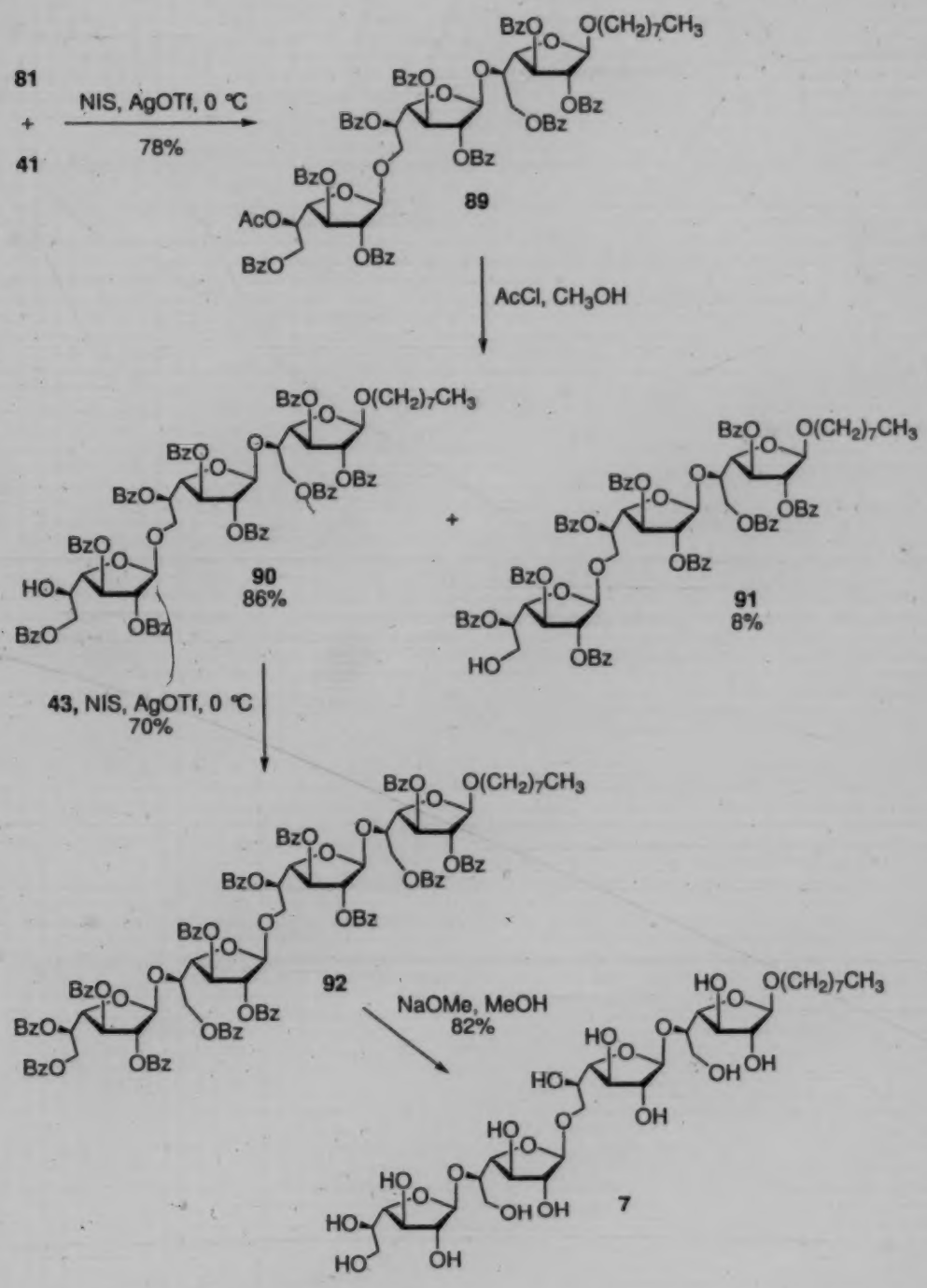
Scheme 30. Synthesis of trisaccharide **6**.

afforded **6** in 89% yield. The structure of **6** was unambiguously confirmed through analysis of its ^{1}H and ^{13}C NMR spectra. The three anomeric hydrogen signals appeared at 5.23 and 4.98 ppm as doublets with $J_{1,2} < 2.0$ Hz and a singlet 5.01 ppm whereas the anomeric carbons were observed at 108.7, 108.1 and 108.0 ppm.

2.6.3. Synthesis of Tetrasaccharides 7 and 8.

The synthesis of tetrasaccharide **7** (Scheme 31) involved a building block, disaccharide **81**, which had been used in the synthesis of the trisaccharide **5** (see Scheme 29 above). The first step in the synthesis of **7** was the coupling of **81** with the 5-*O*-acetyl-2,3,6-tri-*O*-benzoyl-protected thioglycoside **41**, upon activation with NIS and AgOTf, which furnished trisaccharide **89** in 78% yield. The presence of a third β-galactofuranosyl residue was verified by comparison of the ^{1}H NMR spectrum **89** with that of **81**; an additional anomeric hydrogen resonance, a singlet at 5.21 ppm, was present in the spectrum of **89**, as was a resonance corresponding to the acetate methyl group (1.96 ppm).

The acetyl group was then selectively deprotected upon treatment with methanolic HCl to provide trisaccharide **90** in 86% yield. Migration of the benzoyl group at O-6'' to O-5'' was also observed leading to an 8% yield of trisaccharide **91**, whose structure was established as described earlier for **84**. The trisaccharide acceptor **90** was then glycosylated with thioglycoside **43** to obtain tetrasaccharide **92** in 70% yield. Subsequent debenzoylation of **92** afforded tetrasaccharide **7** in 82% yield. In the ^{1}H NMR spectrum of **7**, the resonances of the anomeric hydrogens were observed as three doublets at 5.23 ppm ($J_{1,2}$ = 1.8 Hz), 5.22 ppm ($J_{1,2}$ = 2.0 Hz) and 4.96 ppm ($J_{1,2}$ = 1.0 Hz) as well as a

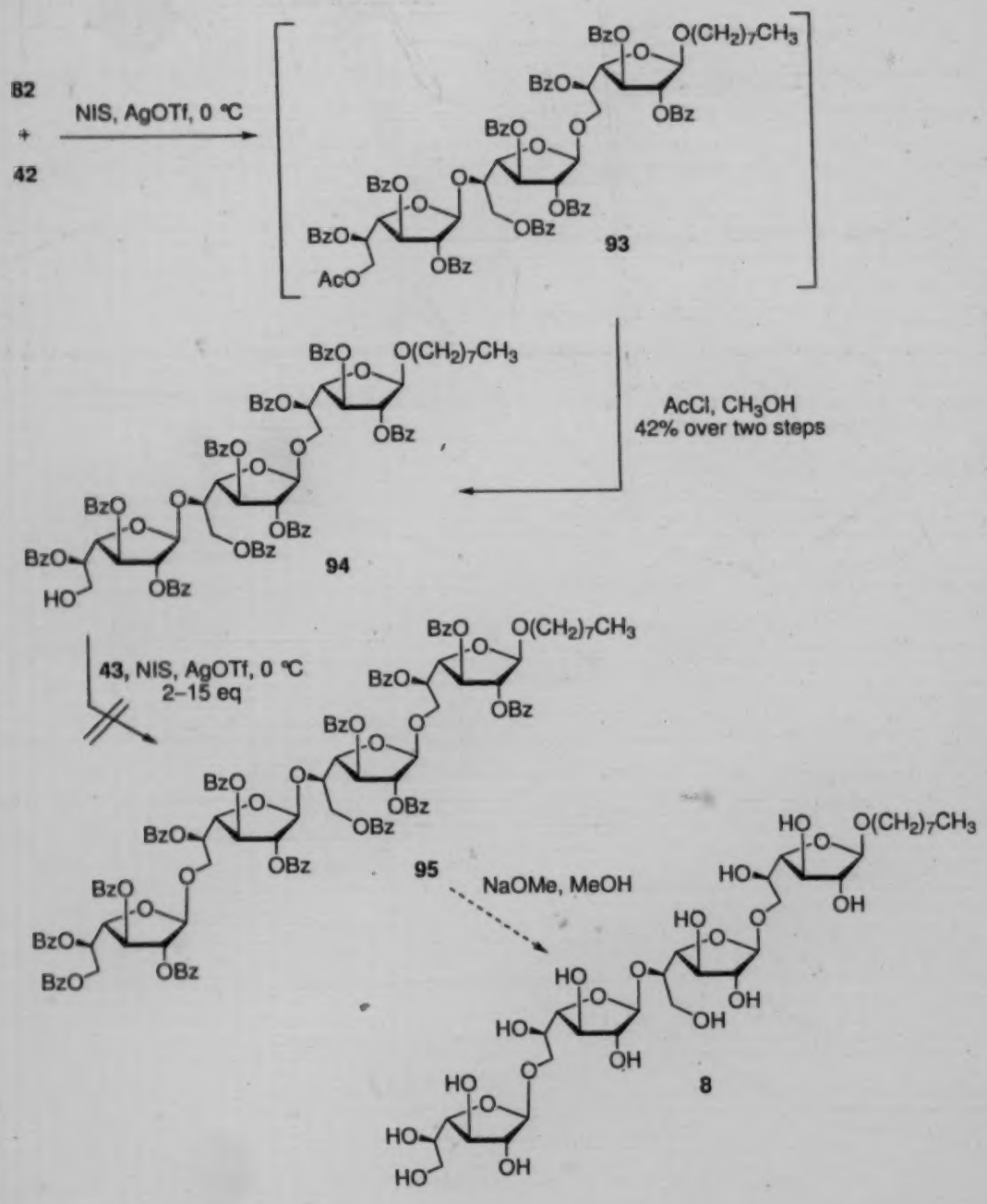


Scheme 31. Synthesis of tetrasaccharide **7**.

singlet at 5.01 ppm. These chemical shifts are in line with the trend outlined above and support a tetrasaccharide with two β-(1→5)-linked Gal*f* residues, a single β-(1→6)-linked Gal*f* residue and one Gal*f* residue β-linked to the octyl aglycone. Further support for the structure came from the ^{13}C NMR spectrum of **7**, which showed four anomeric carbons at 110.6, 109.9, 109.8, and 109.7 ppm, consistent with the β-Gal*f* stereochemistry.

The same general strategy used to prepare **7** was also explored for the preparation of tetrasaccharide **8** (Scheme 32), but problems were encountered. To implement this approach, disaccharide **82**, prepared as shown in Scheme 30, was first coupled with thioglycoside **42** to furnish trisaccharide **93**. The structure of the expected product was confirmed by the appearance of an additional anomeric hydrogen resonance, seen as a singlet at 5.76 ppm, in the ^{1}H NMR spectrum when compared to the spectrum of disaccharide **82**. It was difficult to purify trisaccharide **93** completely from other reaction by-products using chromatography and therefore it was partially purified and carried onto the next step. Deacetylation of partially purified **93** gave the trisaccharide **94** in 42% yield over the two steps.

However, when **94** was reacted with thioglycoside **43** under standard NIS–AgOTf activation conditions, the expected coupling product (**95**) was not obtained. The reaction gave only the hydrolyzed donor and the unreacted acceptor. This result was unexpected, especially considering that the acceptor is a primary alcohol, which, theoretically, should be very reactive in glycosylation reactions.

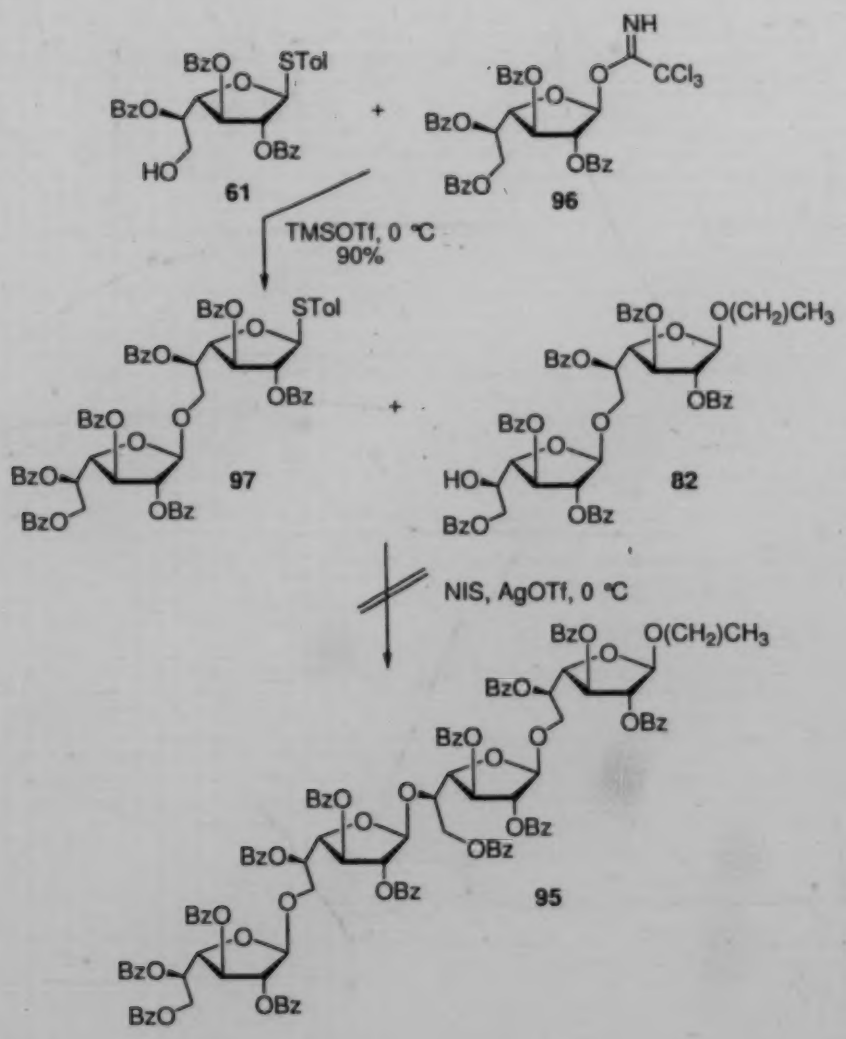


Scheme 32. Attempted synthesis of tetrasaccharide **8** via 1 + 3 coupling strategy.

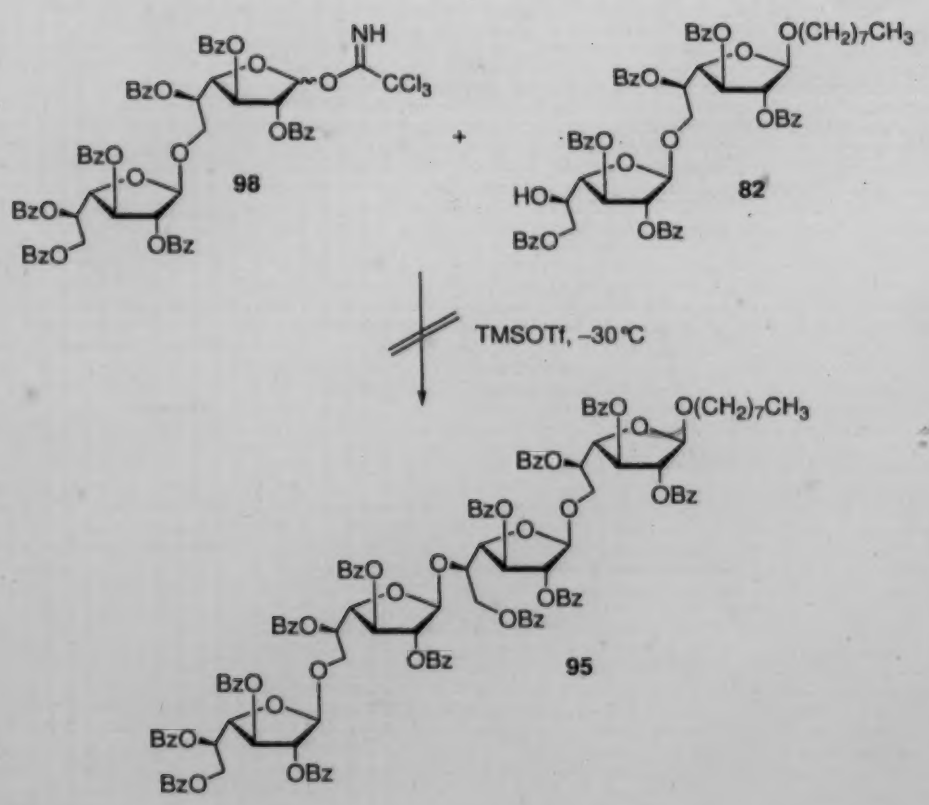
Despite repeated attempts to obtain the desired tetrasaccharide, under a variety of conditions (e.g., changing the number of equivalents of **43** and reaction temperature) no significant amount of the desired product **95** could be isolated.

Faced with this failure, we explored other approaches to tetrasaccharide **95**, and, in turn, the ultimate target **8**. Considering that glycosylation of disaccharide **82** with a thioglycoside **43** (Scheme 30) had proceeded successfully, we investigated the approach outlined in Scheme 33, which involved, as the key step, a 2 + 2 coupling between **82** and disaccharide thioglycoside **97**. Thus, thioglycoside alcohol **61** was reacted with the known tricholoracetimidate[152] **96**, in the presence of TMSOTf, which provided a 90% yield of **97**. Unfortunately, the NIS–AgOTf-promoted glycosylation of **82** with **97** failed to generate any of the desired tetrasaccharide product **95**.

With the failure of the reaction between **82** and **97**, we thought that the use of a different class of donor might give better results (Scheme 34). Therefore, disaccharide thioglycoside **97** was converted into trichloracetimidate **98** in two steps (see section 2.7.3 and Scheme 41 for details). Although the synthesis of **98** was successful, its subsequent reaction with disaccharide alcohol **82** in the presence of TMSOTf was not and none of tetrasaccharide **95** could be isolated. Therefore, this approach was also abandoned.

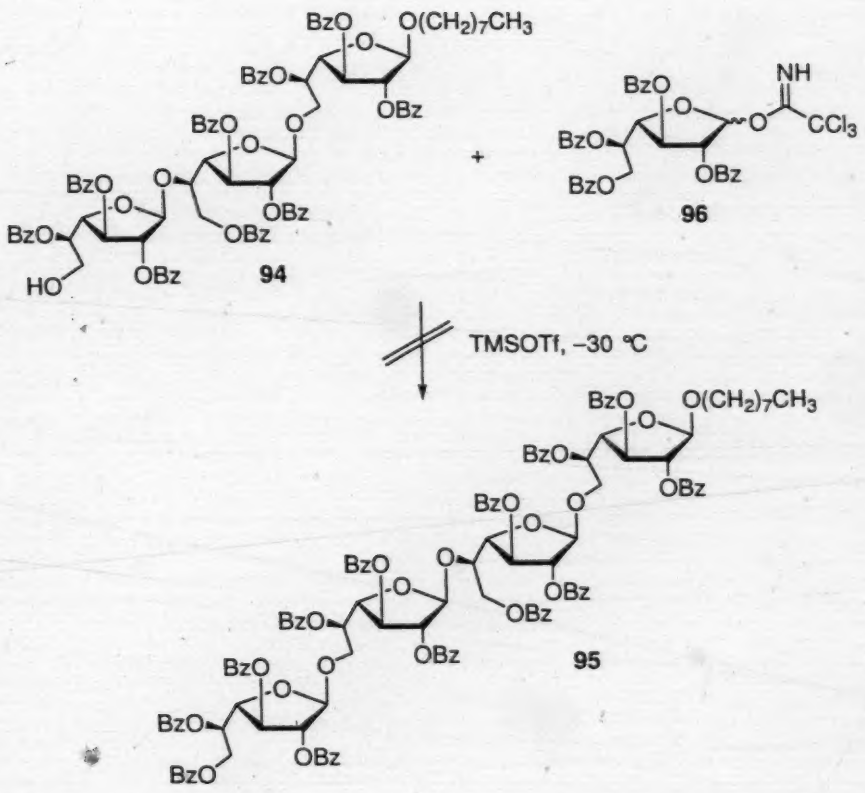


Scheme 33. Attempted synthesis of tetrasaccharide **95** via 2 + 2 thioglycoside coupling.



Scheme 34. Attempted synthesis of tetrasaccharide **95** via 2 + 2 imidate coupling.

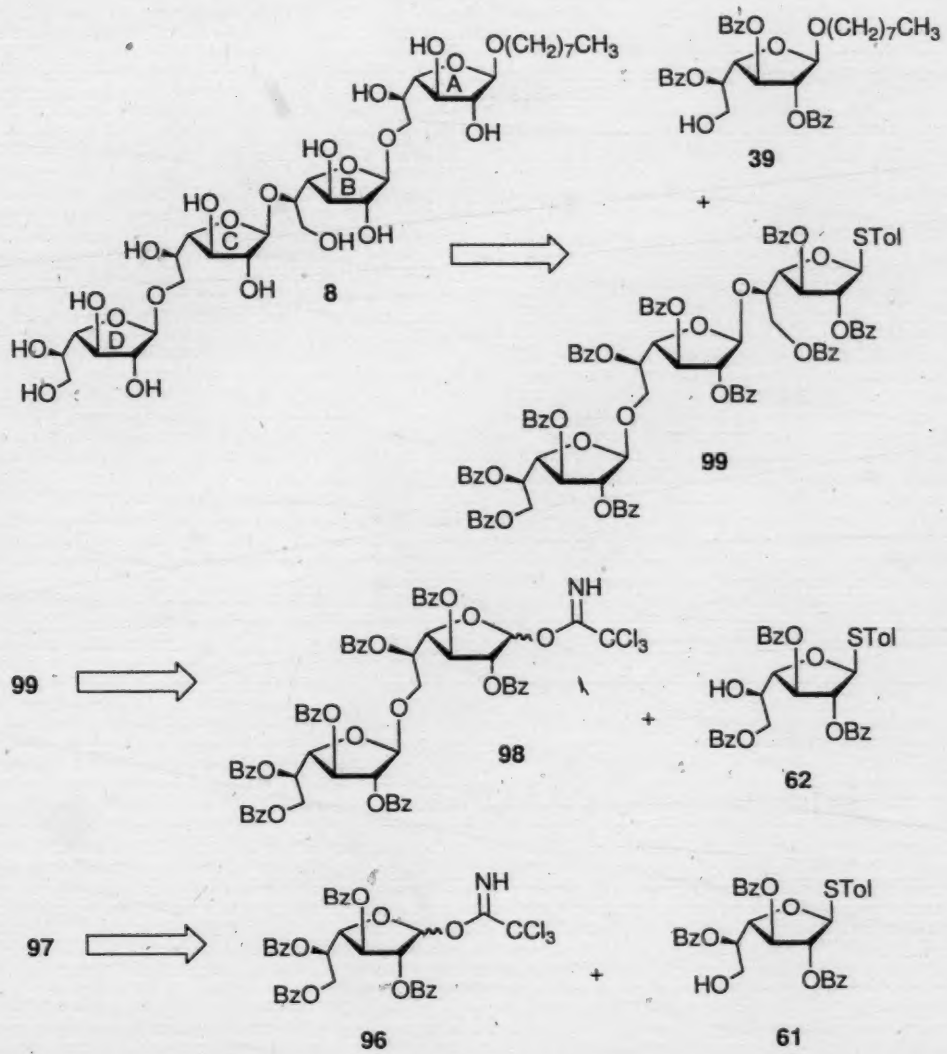
Having been unable to synthesize tetrasaccharide **8** via a 2 + 2 strategy, we next returned to our original 3 + 1 coupling approach (see Scheme 32). We were curious as to if the use of a trichloroacetimidate donor, instead of a thioglycoside, would provide better results (Scheme 35). Frustratingly, activation of trichloroacetimidate **96**[152] by reaction with TMSOTf at –40 °C in the presence of trisaccharide alcohol **94** did not lead to any of the desired tetrasaccharide **95**. Given these cumulative failures, we re-evaluated the general approach to tetrasaccharide **8**, as outlined in the next section.



Scheme 35. Attempted synthesis of tetrasaccharide **95** via 3 + 1 imidate coupling.

2.7 New Approach for the Synthesis of Tetrasaccharides 7 and 8.

We postulated that our failure to prepare tetrasaccharide **8** successfully could be due to conformational changes in the disaccharide or trisaccharide acceptors. These conformational changes may have substantially hindered the primary hydroxyl group in the acceptors making it unavailable during glycosylation reaction. Given the difficulties



Scheme 36. Retrosynthetic analysis of tetrasaccharide **8** via alternate strategy.

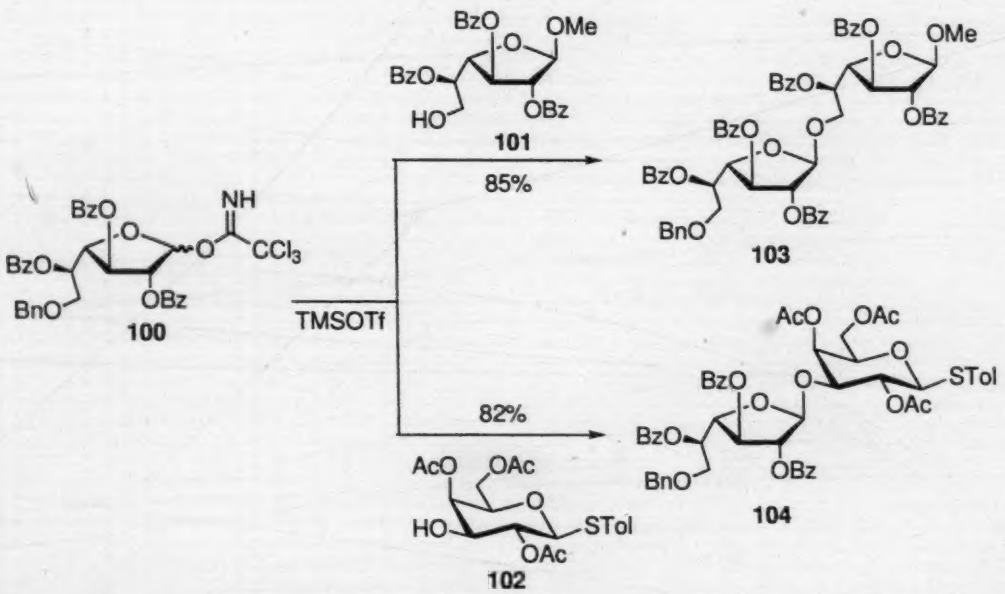
encountered earlier during the synthesis of tetrasaccharide **8**, we opted to design a different synthetic route. The new approach, as outlined in Scheme 36, involved a linear synthesis of the target tetrasaccharide starting from the non-reducing end D and successively extending the length of the oligosaccharide up to the reducing end A.

Based on this retrosynthetic analysis, tetrasaccharide **8** could be prepared through coupling of trisaccharide thioglycoside donor **99** and octyl glycoside acceptor **39**. The formation of trisaccharide **99** could be achieved via glycosylation between imidate donor **98** and acceptor **62**. Donor **98** could, in turn, be synthesized through conversion of the corresponding thioglycoside, formed from coupling of imidate **96** and acceptor **61**, to the glycosyl trichloroacetimidate. We also decided to explore the same approach for the synthesis of the other tetrasaccharide target, **7** (retrosynthesis not shown), so that both strategies could be compared.

2.7.1 Synthesis of Trichloroacetimidate 96.

The trichloroacetimidate method of glycosylation introduced by Schmidt has been extensively used in preparation of glycopyranosides.[153] The use of the methodology in the synthesis of furanosides has been less studied. However, the preparation and glycosylation reactions of protected mannofuranosyl[154] and ribofuranosyl trichloroacetimidates[155] have been reported as has the use of galactofuranosyl trichloroacetimidate **96**.[152] Scheme 37 shows an example of the first reported use of a galactofuranosyl trichloroacetimidate donor for the synthesis of disaccharides containing β-D-galactofuranoside residues.[145] In this example, trichloroacetimidate **100** was reacted with acceptors having free primary (**101**) or secondary hydroxyl groups (**102**) using

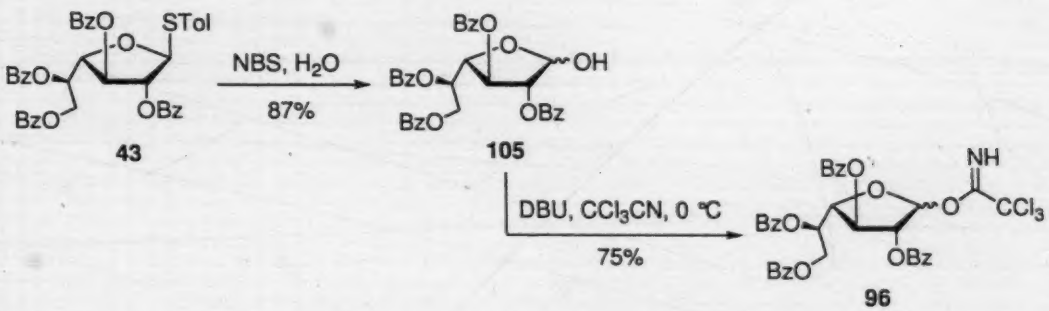
TMSOTf as promoter to give the β-linked disaccharide derivatives **103** and **104**. Furanose trichloroacetimidate donors have since been successfully used for the synthesis of a number of galactofuranosyl containing compounds.[152,156-160]



Scheme 37. Synthesis of galactofuranosyl disaccharides using trichloroacetimidate **100**.

To utilize **61** and **62** as glycosyl acceptors, it was necessary to prepare trichloroacetimidate **96** from thioglycoside **43** (Scheme 38). Thioglycoside **43** was first hydrolyzed by treating with *N*-bromosuccinimide and water in ethyl acetate at room temperature for three hours to give reducing sugar **105** in 87% yield. Subsequent treatment of intermediate **105** with Cl_3CCN and DBU afforded trichloroacetimidate **96** in 75% yield as a 10.6:1 β:α ratio. In the ^1H NMR spectrum of the product, the anomeric hydrogen for the β-anomer appears at 6.70 ppm and that for α-anomer as a doublet at 6.87 ppm ($J_{1,2}$ = 4.0 Hz), which is consistent with other published data for

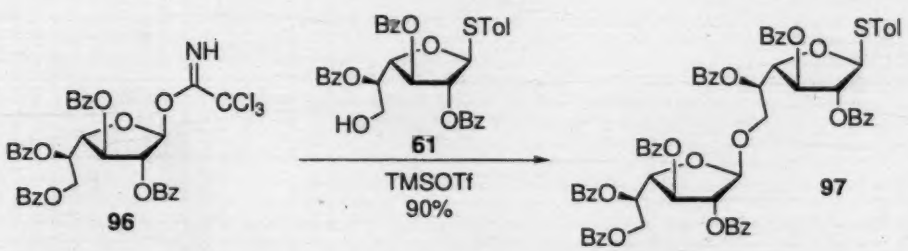
galactofuranosyl trichloroacetimidates.[145,152] However, a disadvantage of using this donor is its instability, which necessitated storage at –20 °C.



Scheme 38. Synthesis of trichloroacetimidate **96**.

2.7.2 Synthesis of Disaccharides 97 and 106.

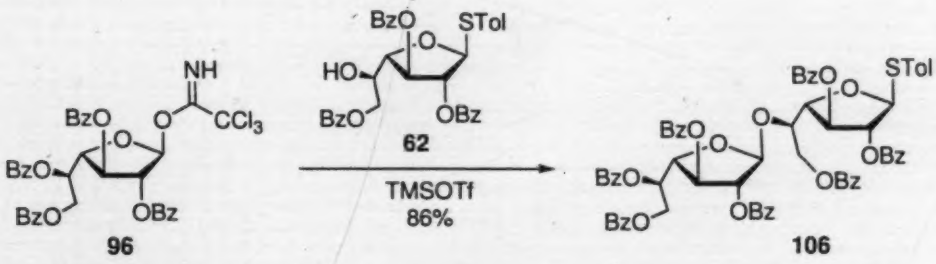
Galactofuranosyl trichloroacetimidate **96** was coupled with acceptor **61** upon activation with TMSOTf to afford disaccharide **97** in 90% yield (Scheme 39). The ^{1}H NMR spectrum of disaccharide **96** showed the chemical shifts for the anomeric hydrogens at 5.78 ppm as a doublet ($J_{1,2}$ = 0.5 Hz) and at 5.38 ppm as a singlet. This confirmed the introduction of the galactofuranosyl moiety at the non-reducing end having



Scheme 39. Synthesis of disaccharide **97** using trichloroacetimidate **96**.

the β-configuration. The structure of the product was further supported by the ^{13}C NMR spectra, which showed the two anomeric carbons at 106.0 and 91.4 ppm.

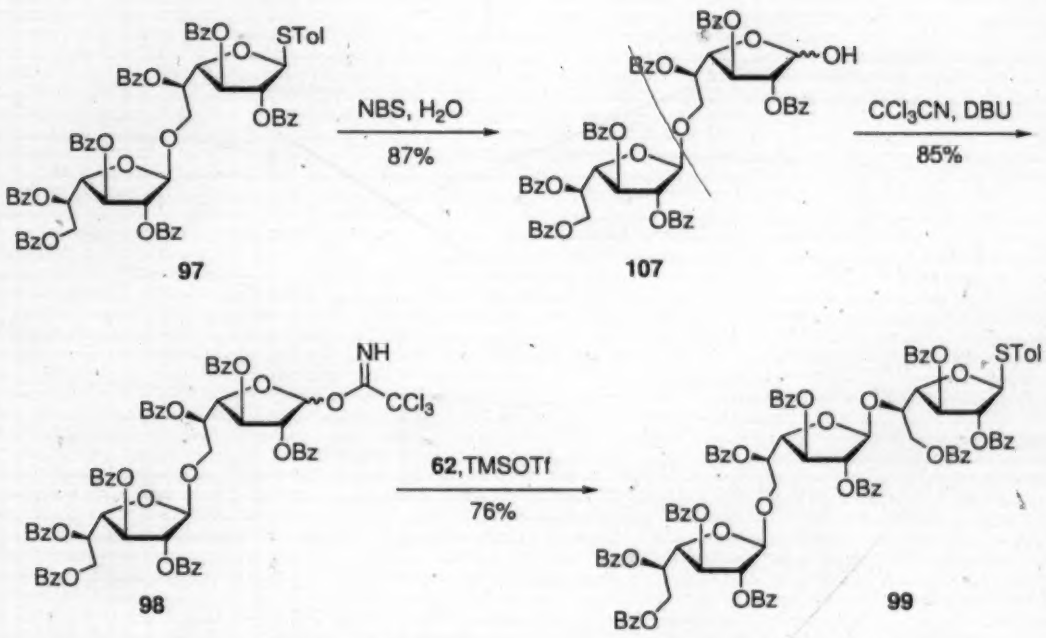
For the anticipated synthesis of tetrasaccharide **7** by the same general strategy, disaccharide thioglycoside **106** was prepared through glycosylation of **62** with imidate donor **96** in the presence of TMSOTf (Scheme 40). The structure of the product could be confirmed using ^{1}H and ^{13}C NMR spectroscopy as outlined for disaccharide **105**.



Scheme 40. Synthesis of disaccharide **106** using trichloroacetimidate **96**.

2.7.3 Synthesis of Trisaccharides 99 and 110.

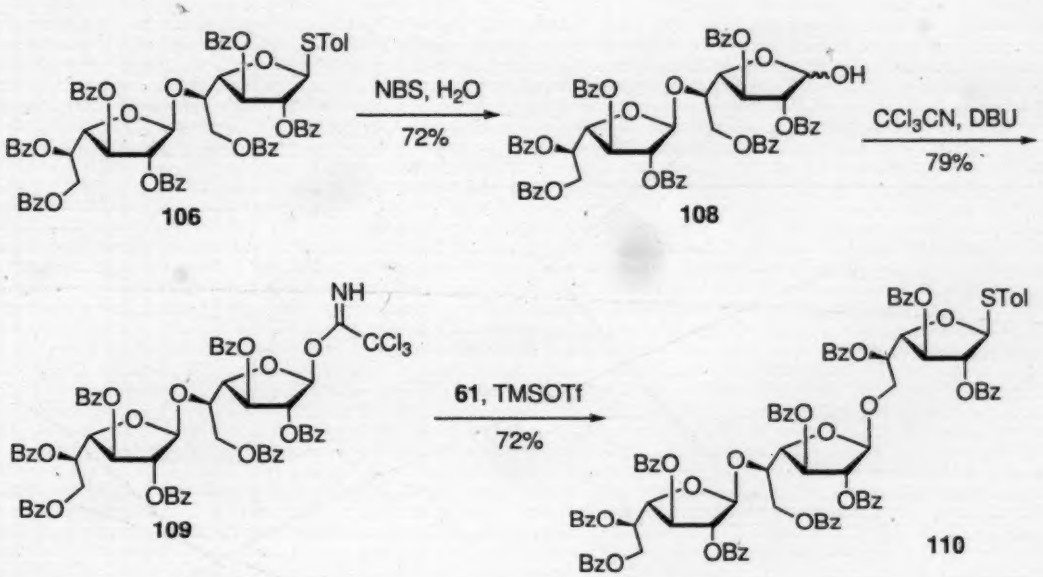
The synthetic route for the preparation of trisaccharide **99**, required for accessing tetrasaccharide **8,** is illustrated in Scheme 41. Starting with thioglycoside **97**, treatment with NBS and H_2O in ethyl acetate gave lactol **107** in 74% yield. The cleavage of the aglycone from **97** was apparent by the absence in the ^{1}H NMR spectrum of **107** of a singlet around 2.2 ppm, arising from *p*-thiotolyl methyl group. In addition, the ^{13}C NMR spectrum of **106** showed the two anomeric carbons resonating at 106.5 ppm (C-1β) and 100.6 ppm (C-1α), downfield from the C-1 resonance in the spectrum of **96**, thus indicating replacement of the sulfur at the anomeric centre with oxygen.



Scheme 41. Synthesis of trisaccharide **99**.

Treatment of intermediate **107** with Cl_3CCN and DBU gave trichloroacetimidate **98** as a 9:1 β:α ratio of anomers in 85% yield. The ^1H NMR spectrum showed the H-1 signal for the two anomers having shifts of 6.78 and 6.85, which arise from the β and α anomers, respectively. Glycosylation of acceptor **62** with disaccharide imidate **98** upon activation with TMSOTf gave trisaccharide **99** in 87% yield. Inspection of the ^1H NMR spectrum showed the three anomeric hydrogens as singlets at 5.79, 5.70 and 5.21 ppm while in the ^{13}C NMR spectrum, the anomeric carbons resonated at 106.7, 105.8 and 91.1 ppm. The appearance of the chemical shift of the third anomeric carbon (δ_C = 91.1 ppm) upfield relative to *O*-glycoside moieties is indicative of the presence of the thiol aglycon at the reducing end.

The synthesis of tetrasaccharide **7** by this new approach required the synthesis of trisaccharide **110**, which was achieved via an analogous strategy (Scheme 42). Thus, thioglycoside **106** (prepared as shown in Scheme 40) was hydrolyzed using NBS and H_2O in ethyl acetate to give disaccharide **108** in 72% yield. Subsequent treatment of **108** with Cl_3CCN and DBU provided imidate **109** in 79% yield. This disaccharide donor **109** was then coupled with acceptor **61** in the presence of a catalytic amount of TMSOTf to afford trisaccharide **110** in 72% yield. The three anomeric hydrogens were observed as a doublet at 5.74 ppm ($J_{1,2}$ = 1.7 Hz) and two singlets at 5.81 ppm and 5.28 ppm, as would be expected for a trisaccharide having β-galactofuranosyl moieties. The ^{13}C NMR spectrum also showed the anomeric carbons at 105.9, 105.2 and 91.3 ppm, which is also consistent with the proposed structure.

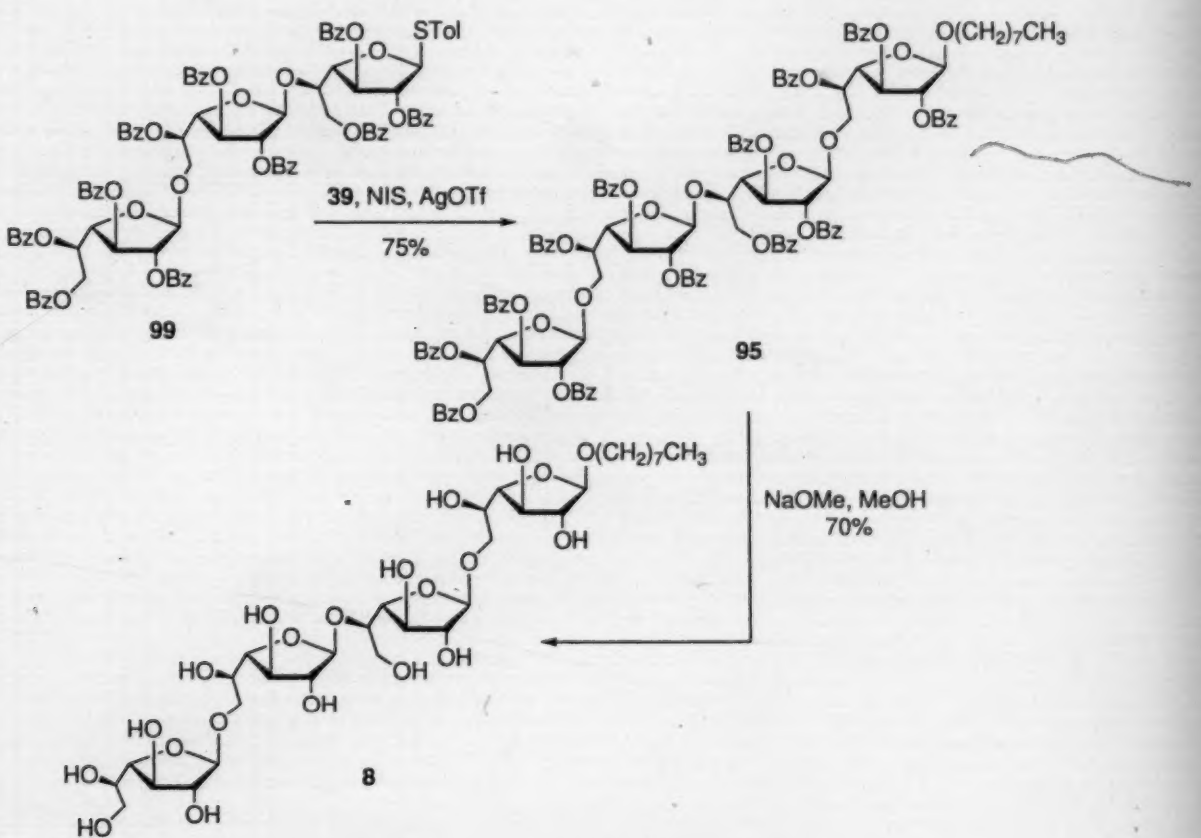


106 108 109 110

Scheme 42. Synthesis of trisaccharide **110**.

2.7.4 Synthesis of Tetrasaccharides 7 and 8.

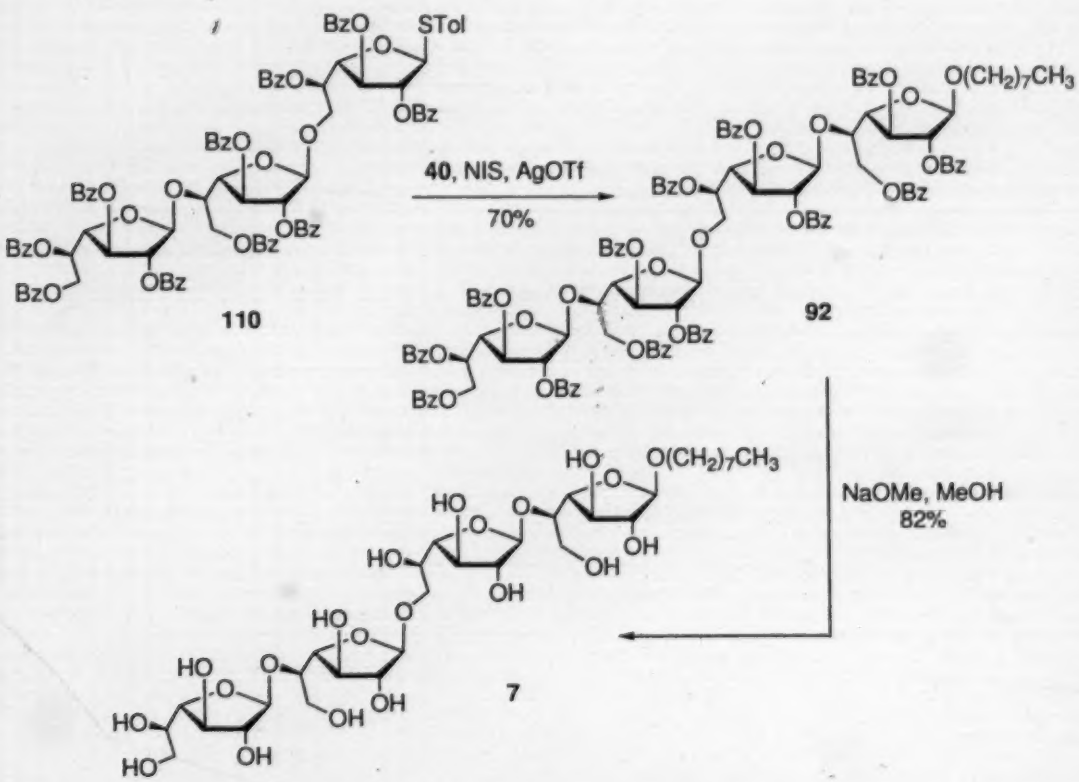
With trisaccharide thioglycosides **99** in hand it was finally possible to synthesize the elusive tetrasaccharide **8** as outlined in Scheme 43. Glycosylation of octyl 2,3,5-tri-*O*-benzoyl-β-D-galactofuranoside (**39**) with trisaccharide **99** in the presence of NIS and AgOTf gave the perbenzoylated tetrasaccharide **95** in 75% yield. Thus, by "inverting" the sequence or reactions, by adding the monosaccharides from the non-reducing to the reducing end, it was possible to access **95** successfully, while the traditional "reducing to non-reducing" approach failed. Subsequent global deprotection of all the benzoyl groups



Scheme 43. Synthesis of tetrasaccharide **8**.

upon treatment with sodium methoxide in methanol gave tetrasaccharide **8** in 70% yield. The ^{1}H NMR spectrum showed the four anomeric hydrogens as singlets at 5.21, 5.03, 5.01 and 4.98 ppm while the four anomeric carbon signals appeared at 108.8, 108.7, 108.0, and 107.9 ppm in the ^{13}C NMR spectrum. All these data are consistent with the structure of tetrasaccharide **8**, which contains two β-(1→6) Gal*f* linkages in addition to one β-(1→5) Gal*f* linkage and a fourth Gal*f* residue β-linked to the octyl aglycone.

With this new effective synthetic route in place for the synthesis of **8**, we proceeded to prepare tetrasaccharide **7** using the same approach as outlined in Scheme 44. With donor **110** in hand (Scheme 42), it was reacted with octyl 2,3,6-tri-*O*-benzoyl-



Scheme 44. Synthesis of tetrasaccharide **7**.

β-D-galactofuranoside (**40**) to afford the perbenzoylated tetrasaccharide **92** in 70% yield; debenzoylation using sodium methoxide in methanol gave **7** in 82% yield. The NMR spectrum of this material was identical to that obtained by the other approach (Scheme 31).

While in the case of **7**, both approaches gave the target in comparable yield and number of steps the "non-reducing to reducing end" strategy may have advantages in the synthesis of large galactofuranosides. This strategy relies on glycosylation reactions with monosaccharide acceptors, which may be less prone to conformational effects that reduce the nucleophilicity of the alcohol. As the size of the chain increases such conformational effects may be expected to increase.

2.7.5 One-pot Syntheses of Trisaccharides 5 and 6.

The preparation of complex oligosaccharides often requires tedious and multiple selective protection and deprotection steps thereby increasing the overall length of the synthesis. A specific example involves sequential and selective protection and deprotection of the intermediates leading to the synthesis of decenyl glycoside of β-D-Gal*f*-(1→5)-β-D-Gal*f*-(1→6)-β-D-Gal*f* and β-D-Gal*f*-(1→6)-β-D-Gal*f* (1→5)-β-D-Gal*f*.[158] The same issues were also seen during the synthesis of oligosaccharides **5–8**. Because of these limitations, there has been considerable interest in the development of one-pot glycosylation strategies.[147,161-164]

In one-pot strategies, the glycosylation partners are carefully chosen such that the product of one reaction can, without any purification, be used in a subsequent glycosylation reaction. A number of elegant one-pot glycosylation methods have been

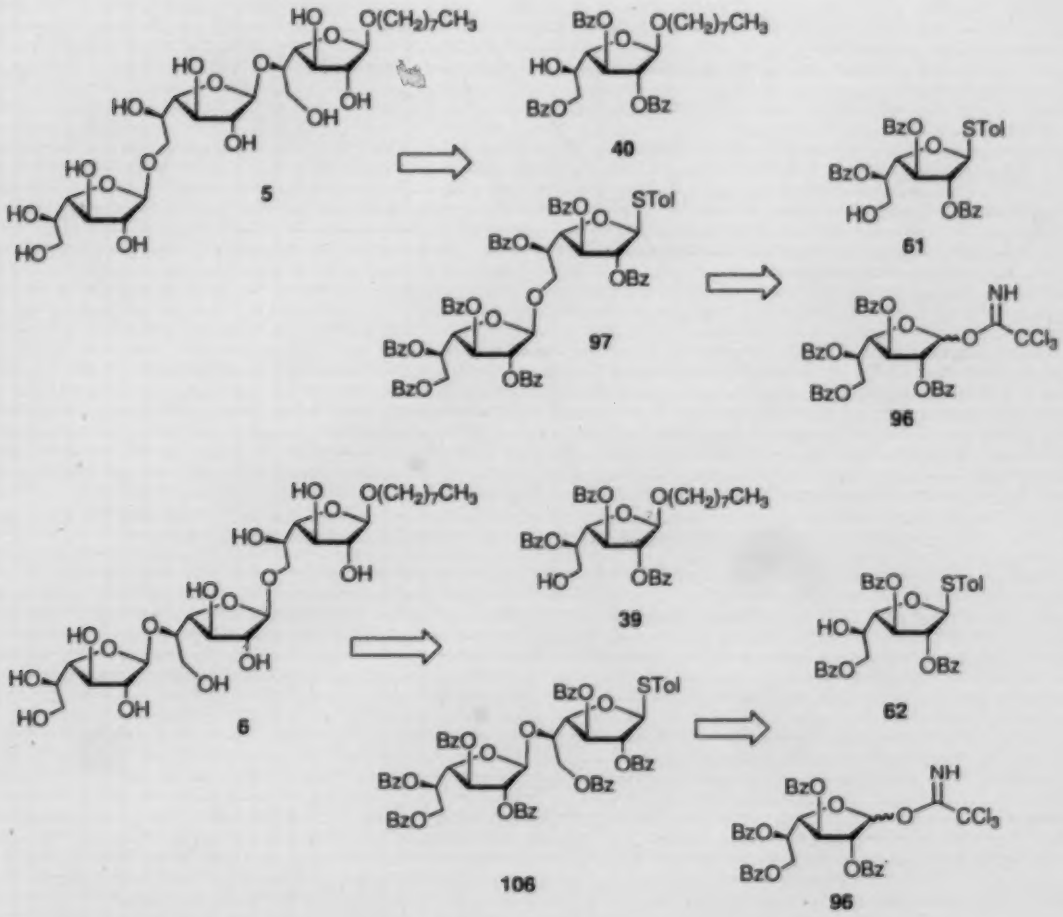
developed and many oligosaccharides have been synthesized using this approach, including the Le^y,[165] fucosyl GM1,[148] and Globo H antigens.[149] This strategy was of interest to us given the tedious nature of the building block preparation required for the synthesis of the oligosaccharides as described above. We therefore developed a one-pot glycosylation strategy for the preparation of trisaccharides **5** and **6**.

It was envisioned that a one-pot glycosylation strategy using trichloroacetimidate **96** and thioglycosides **61** and **62** would be a practical and efficient way of preparing the trisaccharides **5** and **6**. A one-pot strategy using a combination of trichloroacetimidate and thioglycoside donors has been previously used in the synthesis of complex oligosaccharides.[150,166,167] To date, the only furanose-containing oligosaccharide that has been synthesized using a one-pot approach is the trisaccharide α-D-Ara*f*-(1→5)-[β-D-Gal*f*-(1→6)]-D-Gal*f*, another fragment present in mycobacterial cell wall arabinogalactan.[168] In that synthesis, two trichloroacetimidate donors were used and a 1,2-*O*-isopropylidene-protected galactofuranose derivative was the acceptor.

2.7.6 Retrosynthetic Analysis for One-pot Syntheses of Trisaccharides 5 and 6.

The retrosynthetic analysis used for the preparation of trisaccharides **5** and **6** is summarized in Scheme 45. We envisioned that trisaccharide **5** could be synthesized through coupling of thioglycoside disaccharide donor **97** and octyl glycoside acceptor **40**. Donor **97** was, in turn, to be prepared via glycosylation of thioglycoside acceptor **61** with trichloroacetimidate donor **96**. Similarly, trisaccharide **6** could be formed via coupling of disaccharide donor **106** with octyl glycoside acceptor **39**. The disaccharide donor **106** could also be prepared through a coupling reaction between trichloroacetimidate donor

96 and thioglycoside acceptor **62**. In these examples, trichloroacetimidate **96** was used as the first donor for both target trisaccharides with either thioglycoside **61** or **62** serving as the acceptor. Thus, the reactivity between the two possible donors, trichloroacetimidate **96** and thioglycosides **61** or **62**, could be differentiated by utilizing TMSOTf as the activator. Subsequent activation of thioglycoside **96** or **106** using another activator, NIS–AgOTf, and coupling with octyl glycoside acceptor **40** or **39** would then provide trisaccharides **5** and **6** in one-pot.



Scheme 45. Retrosynthetic analysis for one-pot syntheses of trisaccharides **5** and **6**.

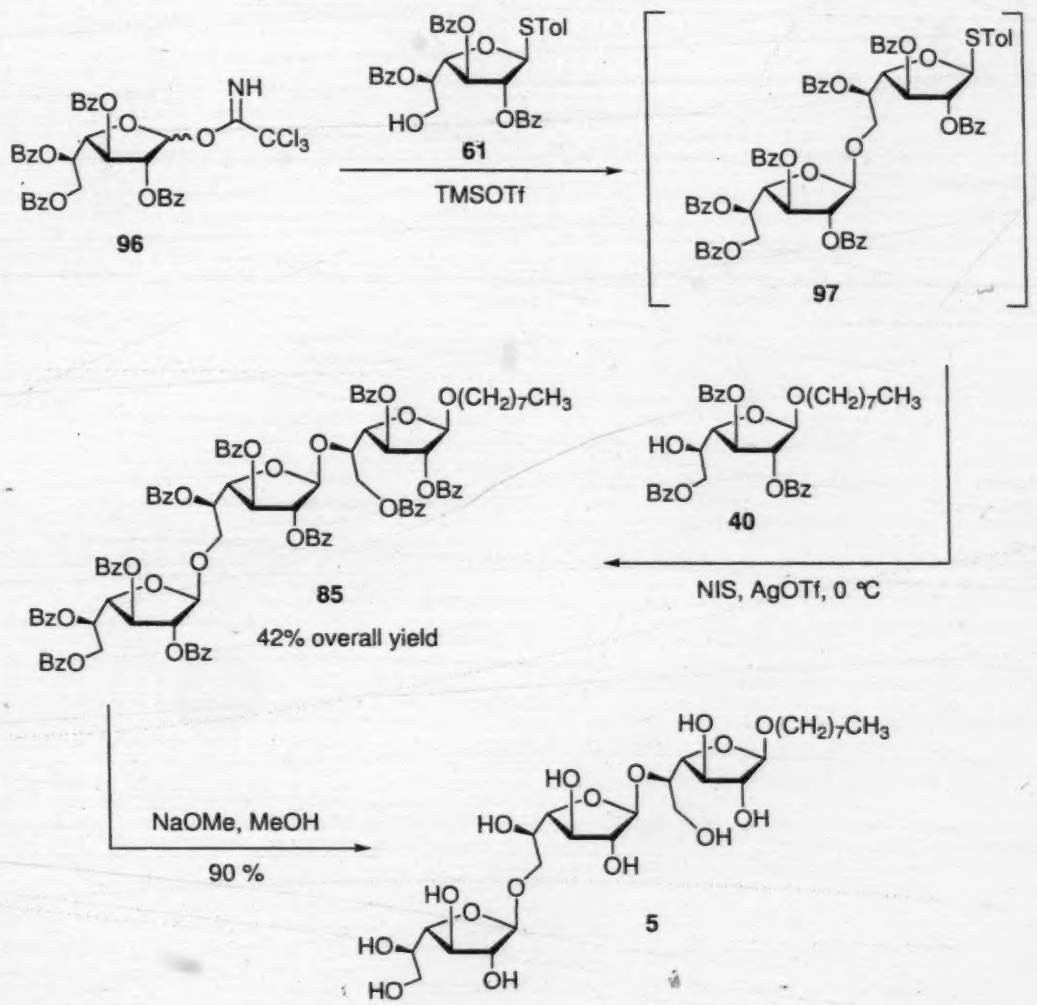
The advantages of this one-pot method include: (1) ease of accessibility of the monosaccharide building blocks, such as trichloroacetimidate **96** and thioglycosides **61** and **62**, (2) *in situ* generation of TfOH in the first glycosylation step, which facilitates the liberation of iodonium ion from NIS in addition to the catalytic amount of AgOTf used in the second glycosylation step, and, (3) elimination of the need for selective deprotection steps on oligosaccharide intermediates, which was a feature of our earlier route to these targets (Schemes 29 and 30). These reactions resulted in varying degrees of benzoyl migration between O-5 and O-6 leading to "dead-end" by-products.

2.7.7 One-pot Syntheses of Trisaccharides 5 and 6.

The implementation of the one-pot glycosylation approach to trisaccharide **5** is shown in Scheme 46. The first step involved the coupling of glycosyl trichloroacetimidate **96** and thioglycoside acceptor **61** using a catalytic amount of TMSOTf as the promoter. While the thioglycoside moiety in **97** is stable to TMSOTf-promoted activation, addition of a second promoter system, NIS–AgOTf, to the reaction mixture resulted in activation of the thioglycoside and its coupling to the second acceptor (**40**) thus affording the perbenzoylated trisaccharide **85** in 42% overall yield. This yield corresponds to an average yield of ~65% for each glycosylation step. The benzoyl protecting groups were then cleaved under standard Zemplén conditions to provide trisaccharide **5** in 90% yield. The NMR data for **5** obtained by this method was identical to those prepared from the material as outlined in Scheme 29.

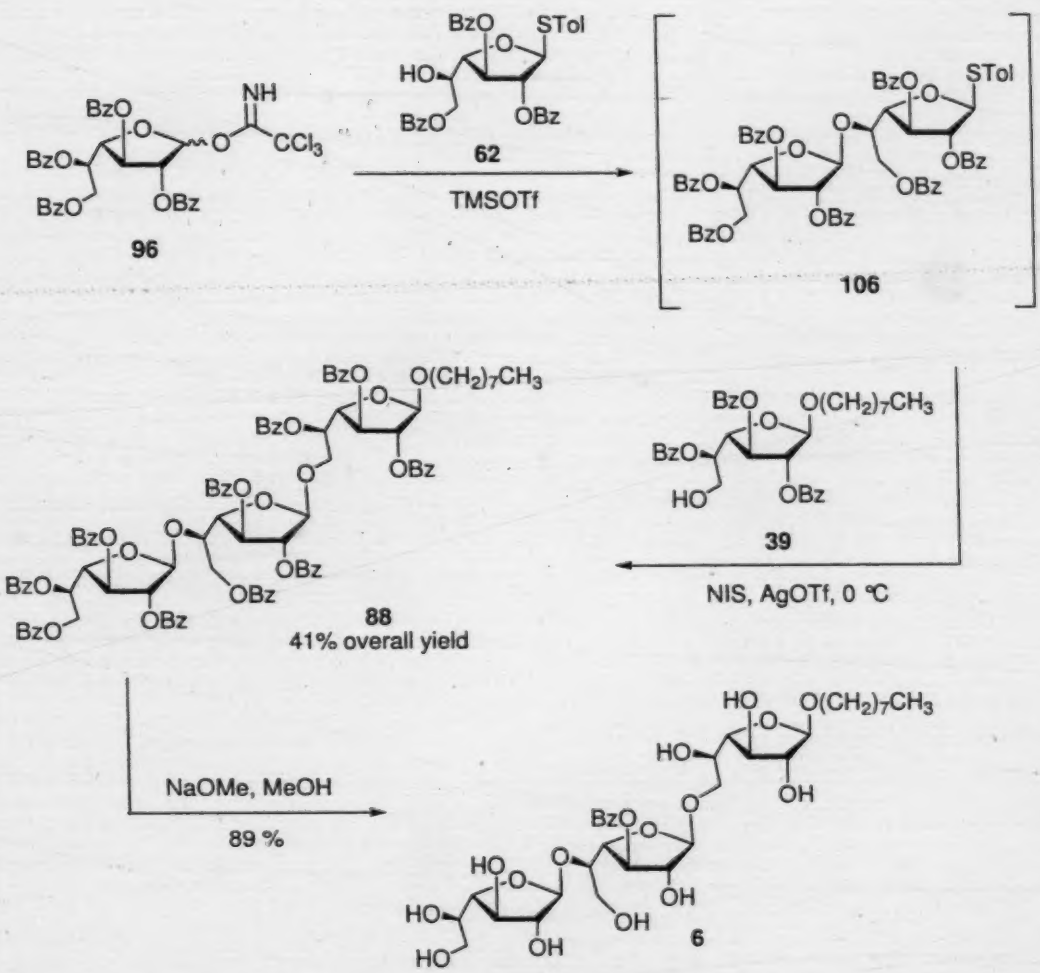
Although the formation of **5** by this process strongly suggests that disaccharide **97** was formed as an intermediate, we endeavoured to prove this. This was achieved by

carrying out the reaction and then stopping the reaction sequence after the first step and showing that the purified product was identical to disaccharide **97** synthesized previously (Scheme 39).



Scheme 46. One-pot synthesis of trisaccharide **5**.

The synthesis of trisaccharide **6** was also performed using the same strategy (Scheme 47). Thus, trichloroacetimidate **96** was coupled with thioglycoside acceptor **62** in the first glycosylation step upon activation with TMSOTf. This was then followed by the addition of octyl glycoside **39** and the NIS–AgOTf promoter to the reaction mixture. This resulted in the formation of trisaccharide **88** in 41% overall yield over two steps. Global deprotection of the perbenzoylated trisaccharide **88** under standard Zemplén



Scheme 47. One-pot synthesis trisaccharide **6**.

conditions then gave trisaccharide **6** in 89% yield. The structure of **6** was validated upon comparison of its NMR data with that obtained from the same trisaccharide prepared using method shown in Scheme 30. As was done in the one-pot synthesis of **5**, in a separate experiment the proposed disaccharide intermediate **106** was isolated, characterized and proven to be identical to **106** prepared as shown in Scheme 40.

The use of one pot glycosylation strategy in the preparation of trisaccharides **5** and **6** in good yield clearly demonstrated that this approach is comparable or even superior to that of the synthetic approach detailed in Scheme 29 and 30. Not only did the one pot strategy circumvent the common pitfalls of the traditional strategy, such as tedious selective protection and deprotection reactions and the associated purification steps, but it greatly expedited the preparation of the target compounds in only a matter of hours. This advance enabled us to synthesize these oligosaccharides in the multi-milligram scale needed for kinetic studies of GlfT2 to probe its specificity.

2.8. Characterization of GlfT2 using Oligosaccharides 1–8.

With oligosaccharides **1–8** synthesized, they were used to characterize the specificity of recombinant GlfT2, which had been expressed in *E. coli*. by Dr. Natisha Rose, a research associate in our laboratory. The kinetic studies described below (section 2.8.1) were carried out by Dr. Rose, but these results are presented to place the remainder of the synthetic work in this thesis in context. In addition, I played a significant role in characterizing the products generated from these substrates by this recombinant enzyme (Section 2.8.2). The results of these investigations were the subject of a recent paper.[76]

2.8.1. Kinetic Characterization of 1–6.

The assay employed was an adaptation of a radiochemical assay developed previously[74] for measuring GlfT2 activity in a crude mycobacterial membrane preparation (Figure 20). Briefly, the assay involves incubation of an oligosaccharide (e.g., **1–8**), GlfT2, UDP-[^{3}H]Gal*p* and purified UDP-Gal*p* mutase.[169] The mutase enzyme converts UDP-[^{3}H]Gal*p* into UDP-[^{3}H]Gal*f*, which is then transferred to the substrate by GlfT2. Using this assay, oligosaccharides **1–6** (Figure 21) were initially screened for their ability to serve as substrates for GlfT2. The results of this screen are presented in Table 2.

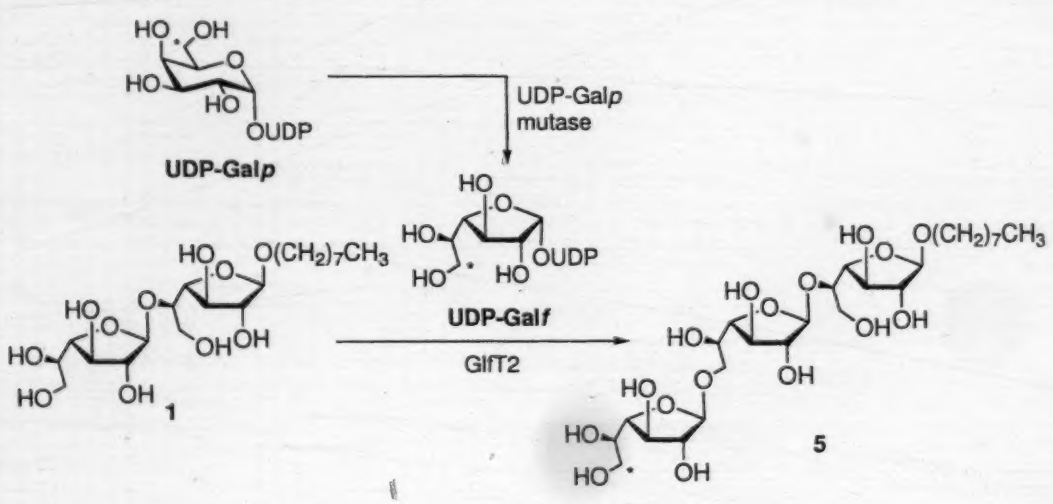


Figure 20. Radiochemical assay for GlfT2.

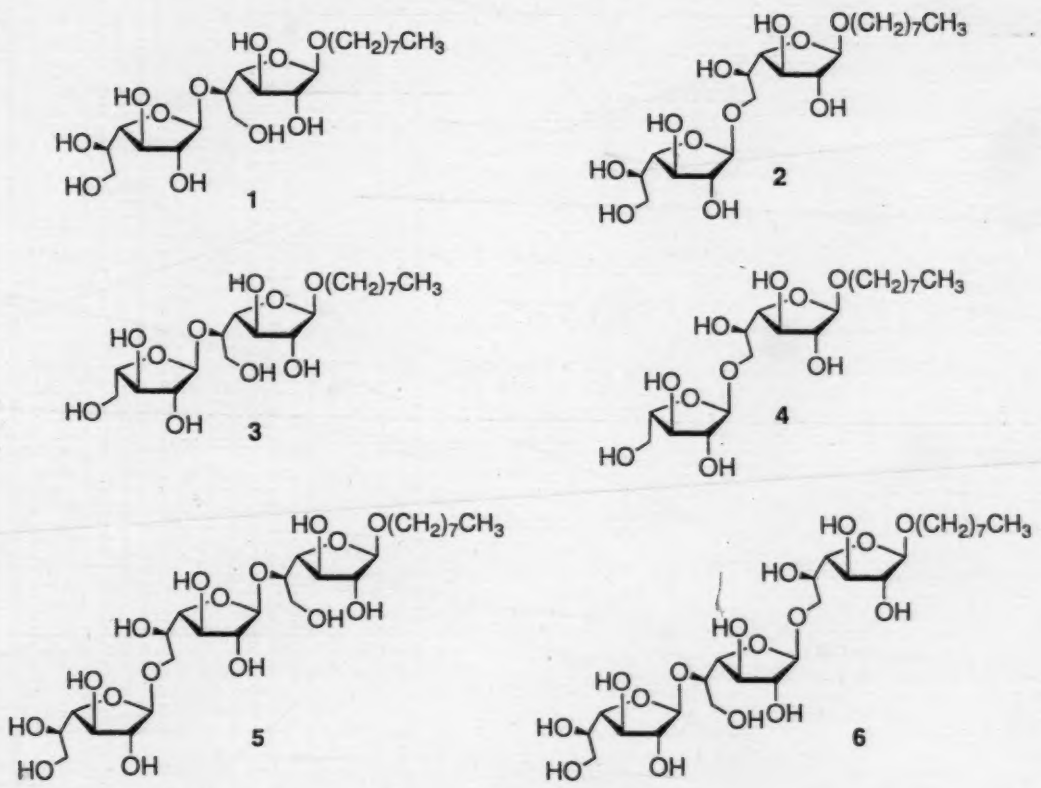


Figure 21. Oligosaccharides **1–6** screened as substrates for GlfT2.

Table 2. Acceptor specificity of recombinant GlfT2 using **1–6**

Acceptor Substrate	Specific Activity[a] (mU/mg)	K_m (μM)	k_{cat} (min^{-1})	k_{cat}/K_m ($\mu M \cdot min)^{-1}$	Relative k_{cat}/K_m
1, (1→5)	0.03	1700 ± 385	0.0016	9.41e-7	1
2, (1→6)	0.03	635 ± 126	0.0039	6.14e-6	6.5
3, (1→5)	0.00	n.d.[b]	n.d.	n.d.	n.d.
4, (1→6)	0.02	n.d.	n.d.	n.d.	n.d.
5, (1→6),(1→5)	0.06	204 ± 34	0.0032	1.57e-5	16.7
6, (1→5),(1→6)	0.09	208 ± 50	0.014	6.73e-5	71.5

[a] Specific activities measured using 1 mM acceptor.
[b] n.d. = not determined.

A number of trends emerged from these data. First, in agreement with previous studies,[74] the β-(1→5) and β-(1→6)-linked disaccharides **1** and **2** are substrates for this bifunctional galactofuranosyltransferase. Secondly, the two trisaccharides, **5** and **6**, are also recognized by the enzyme and are substantially better substrates than **1** and **2** as indicated by the relative k_{cat}/K_m values. Finally, the two disaccharides in which the terminal Gal*f* residues have been replaced with an Ara*f* residues are either not a substrate (**3**) or a poor substrate (**4**).

For the Ara*f*-containing disaccharides, the (1→6)-linked isomer **4** is a substrate for GlfT2, but exhibits lower specific activity than either of the disaccharides containing two Gal*f* residues (**1** or **2**). The other Ara*f*-containing disaccharide, **3**, shows no activity as a substrate. In retrospect, these results are not unexpected. The parent structure for disaccharide **4** is **2**, which are both β-(1→6)-linked; the alternating processive nature of GlfT2 means that if a Gal*f* residue is transferred to these compounds, it will be added to O-5 in the terminal monosaccharide residue. As disaccharide **4** has a hydroxyl group at C-5', it would be expected to be a substrate. However, the reduced activity of **4** compared with **2** suggests that the terminal hydroxymethyl group in **8** is important for recognition and catalysis but that its presence is not essential. In contrast, the parent disaccharide for **3** is the Gal*f*-β-(1→5)-Gal*f* disaccharide **1**. This compound is a substrate for the β-(1→6)-transferase activity of GlfT2. Because the non-reducing residue of **3** lacks C-6 (and also the C-6 hydroxyl group), it is not surprising that this compound is not a substrate.

2.8.2. Characterization of Products obtained from 5 and 6.

To demonstrate that this recombinant enzyme was functioning similar to the wild-type enzyme, it was essential to characterize the products generated by incubation of these compounds with GlfT2. We therefore carried out milligram-scale enzymatic reactions with the two best substrates, trisaccharides **5** and **6**, and characterized the resulting products by mass spectrometry and ^{1}H NMR spectroscopy. After the incubation of each compound with UDP-Gal*p*, GlfT2 and UDP-Gal*p* mutase, the mixture was purified using a C_{18} Sep-Pak cartridge (see experimental), which provided a crude mixture of oligosaccharides. This mixture of products was analyzed by MALDI mass spectrometry (Figure 22).

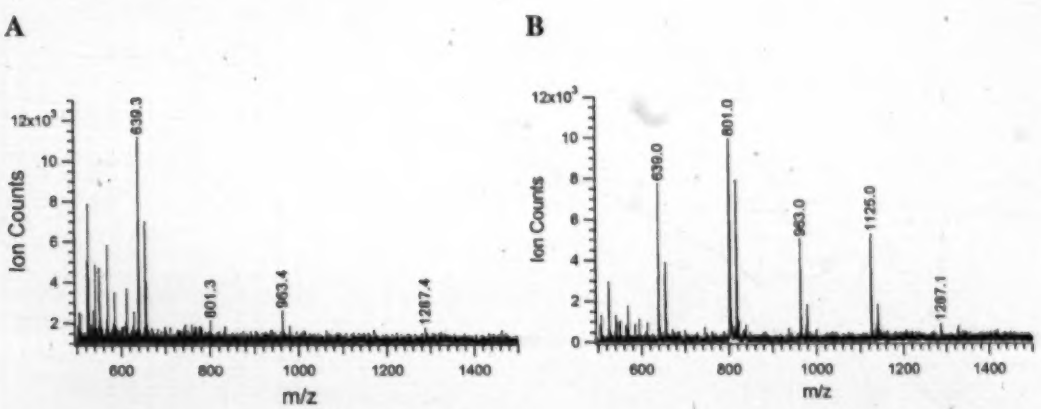


Figure 22. MALDI mass spectra of large-scale incubation mixtures of **5** (A) or **6** (B) with UDP-Gal*f* and GlfT2. The peaks at *m/z* = 639 and 801 are attributed to the sodium adducts of the starting trisaccharides and tetrasaccharide products, respectively, with longer oligomers are found at *m/z* = 963, 1125 and 1287. The "shadow" signals at higher *m/z* are the corresponding potassium adducts.

The spectrum obtained on the products from the reaction with **5** (Figure 22A) shows a signal at *m/z* = 801. This is the mass expected for the sodium adduct of an octyl tetrasaccharide composed of galactofuranose residues; peaks corresponding to longer oligomers are also seen at *m/z* = 963 and 1287. Shown in Figure 5B is the mass spectrum

of the product obtained from the reaction with **6**, which again shows a peak at *m/z* = 801. This result clearly indicates the formation of a tetrasaccharide product, and longer oligomers at *m/z* = 963, 1125 and 1287 are also present. These longer oligomers result from the processive nature of the enzyme as the product of the initial transfer (a tetrasaccharide) is also a substrate for the enzyme.

If the assumption is made that all of the oligosaccharides ionize to similar degrees, the relative abundance of the different oligosaccharides products in these mass spectra suggest that the (1→6)-transferase activity is faster than the (1→5)-transferase activity. For example, in the case of **5** (Figure 22A), after the first Gal*f* residue is added β-(1→5) the resulting product is a substrate for the faster (1→6)-transferase activity. These relative rates are reflected by the fact that the peak corresponding to the tetrasaccharide product (*m/z* = 801) is marginally smaller than that of the pentasaccharide (*m/z* = 963). The apparent absence of a hexasaccharide (*m/z* = 1125), but presence of a heptasaccharide (*m/z* = 1287), is also consistent with these relative rates. These effects are also reflected in the incubations of trisaccharide **6**, in which the products arising from β-(1→5) transfer (the penta- and heptasaccharide, *m/z* = 963 and 1287, respectively) are present in smaller amounts that those resulting from β-(1→6) transfer (the tetra- and hexasaccharides; *m/z* = 801 and 1125, respectively).

Although these experiments allow us to confirm that a homologous series of oligosaccharides was produced by incubation of **5** and **6** with GlfT2, it was not possible to determine the linkages present in these products. Therefore, preparative TLC was used to separate each oligosaccharide mixture and the band corresponding to the tetrasaccharide was extracted from the plate and investigated by ^{1}H NMR spectroscopy.

The anomeric region of the ^{1}H NMR spectra of the purified compounds is shown in Figure 23. In both, one major product is seen, which is contaminated with some impurities. These impurities were later shown by mass spectrometry (data not shown) to be longer oligomers, resulting from incomplete separation of the mixture by TLC.

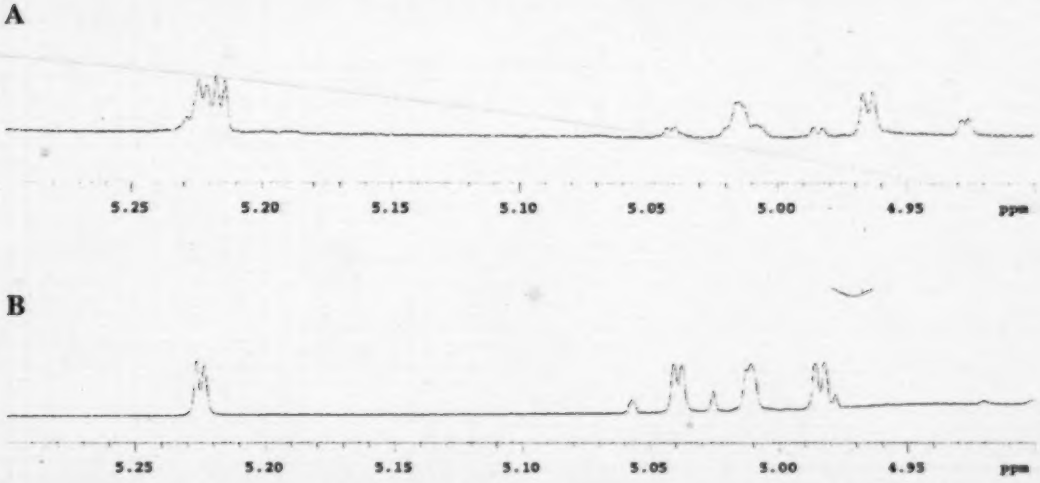


Figure 23. Partial ^{1}H NMR spectra of tetrasaccharides produced from large-scale incubation of **5** (A) or **6** (B) with UDP-Gal*f* and GlfT2. The major signals correspond to anomeric hydrogens in tetrasaccharides **7** and **8**, respectively. Minor resonances arise from longer oligomers that were not effectively separated by preparative TLC.

In the ^{1}H spectrum measured for the tetrasaccharide produced from **5**, four anomeric peaks are present, all with $J_{1,2} < 2$ Hz. The chemical shifts of the resonances are 5.23, 5.21, 5.02 and 4.97 ppm, which, based on the trends described earlier (Section 2.6.1), suggest the presence of two β-(1→5)-linkages, one β-(1→6)-linkage and one β-Gal*f* linkage to the octyl group. This is consistent with the product expected from **5**, tetrasaccharide **7**, and in addition, comparison of these data with those obtained from synthetic **7** show excellent agreement. In the ^{1}H NMR spectrum of the product obtained from **6** (Figure 6B), the four anomeric hydrogens were found at 5.23, 5.04, 5.01 and 4.98 ppm, all as doublets with $J_{1,2} < 2$ Hz or singlets. This suggests the product has a single β-

(1→5)-linkage, two β-(1→5)-linkages and a β-Gal*f* linkage to the octyl group, consistent with the expected tetrasaccharide, **8**. These resonances also match those present in the ^{1}H NMR spectrum of synthetic **8**. Taken together, these structural characterization studies provide strong support that this recombinant protein synthesizes alternating β-(1→6) and β-(1→5)-Gal*f* linkages, as does the wild-type enzyme.

2.9. Formulation of a Hypothesis for Galactan Biosynthesis.

The kinetic parameters presented in Table 2, and structural work reported earlier,[170,171] allowed us to formulate a hypothesis about how mycobacterial galactan is synthesized in vivo and the role of GlfT2 in this process. First, previous structural work on AG has shown that the first two Gal*f* residues are attached to the Rha*p* moiety are linked β-(1→5), not β-(1→6) (Figure 24).[170] In addition, the results presented above show that GlfT2 appears to recognize Gal*f* residues attached either to O-5 or O-6 of another Gal*f* residue. In light of this, we hypothesized that the first Gal*f* residue, attached β-(1→4) to Rha*p*, must be added by a galactofuranosyltransferase other than GlfT2. We viewed this as likely because the putative acceptor, glycoconjugates containing an α-L-Rha*p*-(1→3)-α-D-Glc*p*NAc motif, are structurally very different than the (apparent) preferred acceptors of GlfT2. For the same reasons, we considered it likely that the second Gal*f* residue was also added by an enzyme other than GlfT2. Finally, the data in Table 2 also suggest that GlfT2 does not add the third Gal*f* residue either. This is because the structure of the product after the addition of two Gal*f* residues, β-D-Gal*f*-(1→5)-β-D-Gal*f*-(1→4)-α-L-Rha*p*-(1→3)-α-D-Glc*p*NAc, has as the terminal two residues a β-D-Gal*f*-(1→5)-β-D-Gal*f* motif, which is analogous to **1**, the poorest of all of the substrates

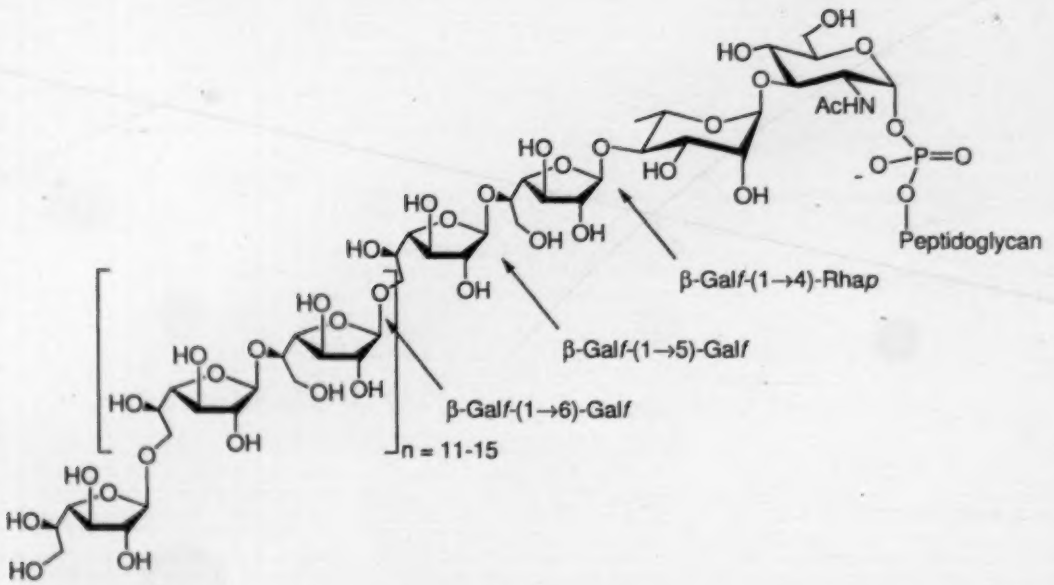


Figure 24. Structure of mycobacterial galactan attached to the linker disaccharide.

we have investigated. We therefore formulated the hypothesis (Figure 25) that three separate enzyme activities were required to produce a pentasaccharide consisting of three β-Gal*f* residues, as well as the Rha*p* and Glc*p*NAc moieties that is then a substrate for GlfT2, which adds the remaining Gal*f* residues in the polymer. To test this hypothesis, we synthesized four additional oligosaccharides, containing the linker disaccharide plus 0–3 Gal*f* residues (Scheme 48).

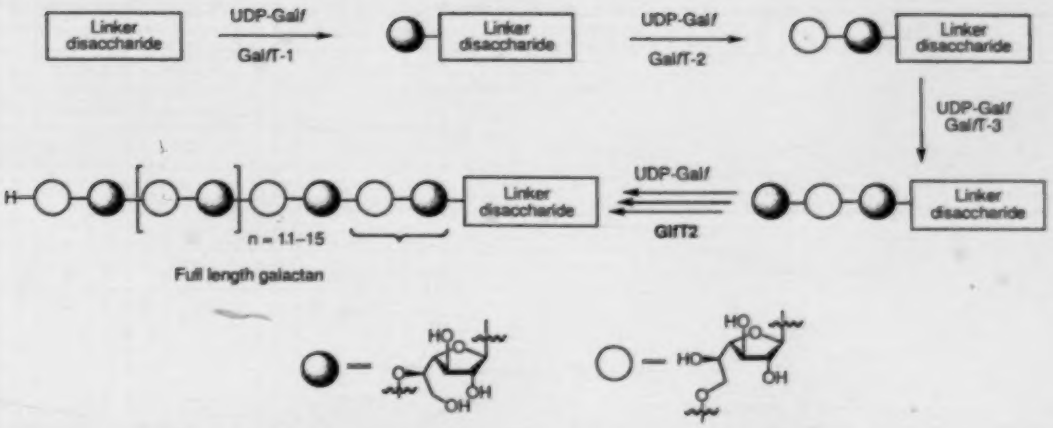
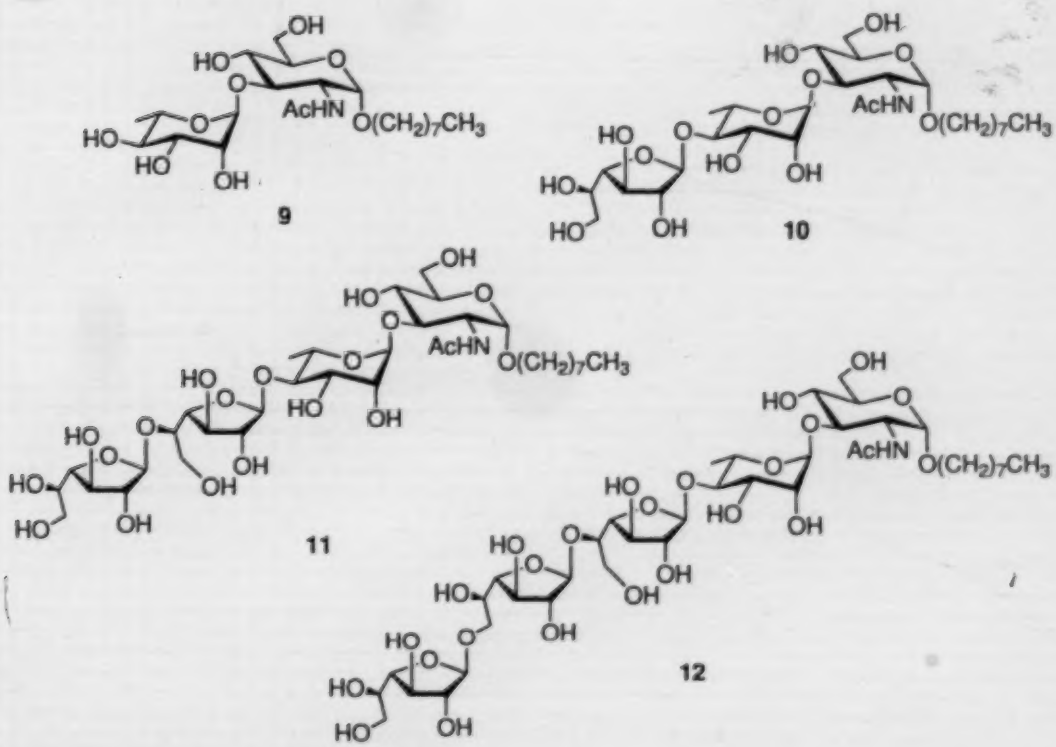


Figure 25. Proposed model for galactan assembly (based on data in Table 2 and available structural data).[170]

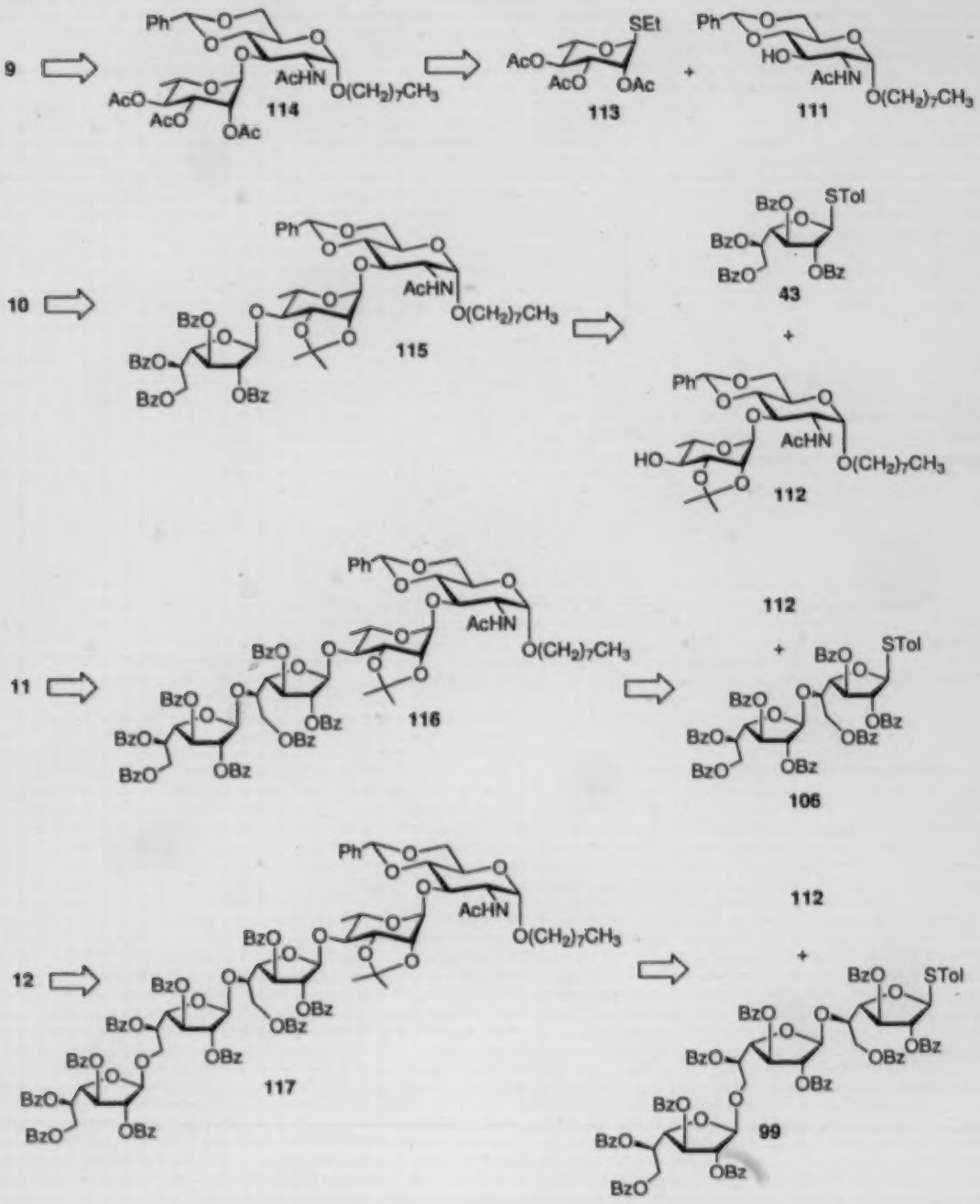


Scheme 48. Oligosaccharide targets **9–12**, which contain the α-L-Rha*p*-(1→3)-α-D-Glc*p*NAc linker disaccharide.

2.10. Synthesis of Oligosaccharides with Linker Disaccharide.

2.10.1 Retrosynthetic Analysis for 9–12.

Scheme 49 shows the retrosynthetic disconnections proposed for the preparation of target compounds **9–12**. The α-L-Rha*p*-(1→3)-α-D-Glc*p*NAc-O$(CH_2)_7CH_3$ (**9**) could be prepared via **114** from the known peracetylated ethyl thioglycoside donor **113**[127] and the benzylidene protected octyl glycoside acceptor **111**, which has also been previously reported.[172] For the synthesis of compounds **10–12**, octyl disaccharide **112**, obtainable from **114**, could be used as the acceptor and the previously prepared mono-, di-, and trisaccharide donors **43**, **106** and **99**, respectively, could be used as the donors. Each of these three thioglycosides could be conveniently used for the coupling reactions to form the protected target compounds **114–117** in a single step. The convergent nature of the route and the simple two-step deprotection sequences involving simultaneous cleavage of the benzylidene acetal and isopopylidene ketal groups followed by debenzoylation make this an efficient synthetic route for obtaining **9–12**.

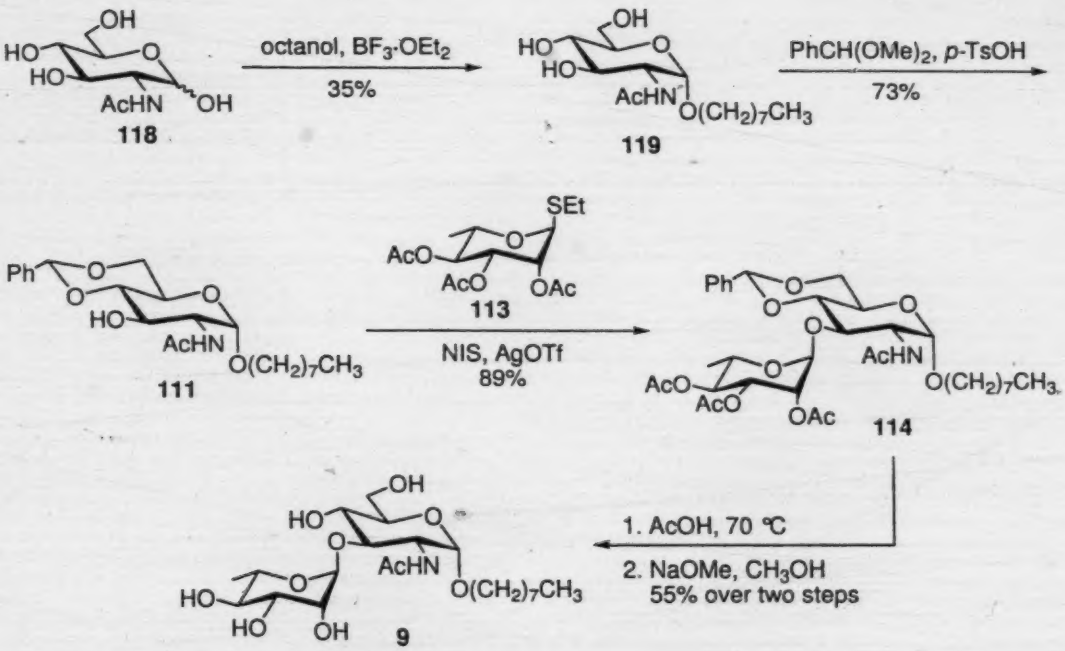


Scheme 49. Retrosynthetic analysis for **9–12.**

2.10.2. Synthesis of Disaccharide 9.

The synthesis of disaccharide **9** started from inexpensive *N*-acetyl-D-glucosamine **118** as outlined in Scheme 50. Several approaches were investigated to prepare octyl 2-acetamido-2-deoxy-α-D-glucopyranoside (**119**). The first involved heating **118** at 100 °C overnight in a mixture of octanol, THF and acidic resin.[172] This reaction, however, gave only recovered starting material. Another approach was based on a reported preparation of phenyl 2-acetamido-2-deoxy-α-D-galactopyranoside from peracetylated *N*-acetyl-D-galactosamine.[173] This required the reaction of peracetylated *N*-acetyl-D-glucosamine with *n*-octanol in the presence of $ZnCl_2$ and $CaSO_4$, but this method also failed to give the acetylated derivative of **119**.

These problems prompted us to consider a reported Fischer glycosylation method,[172] which was done by suspending *N*-acetyl-D-glucosamine in a solution of *n*-octanol in dry acetonitrile with boron trifluoride diethyl etherate (Scheme 50). After an 18-hour reflux, the solution was filtered to remove the unreacted starting material and the desired product, **119**, was obtained in 35% yield after purification of the filtrate by column chromatography. In the ^{1}H NMR spectrum of the product, the resonance for H-1 appeared as a doublet with $J_{1,2}$ = 3.7 Hz, indicating that that **119** had the desired α-stereochemistry. A product with the β-configuration would have appeared as a doublet with a larger $J_{1,2}$ (~ 8.0 Hz). Having developed a robust method for producing **119**, this compound was converted to **111** in 73% yield upon treatment with benzaldehyde dimethyl acetal and *p*-toluenesulfonic acid.



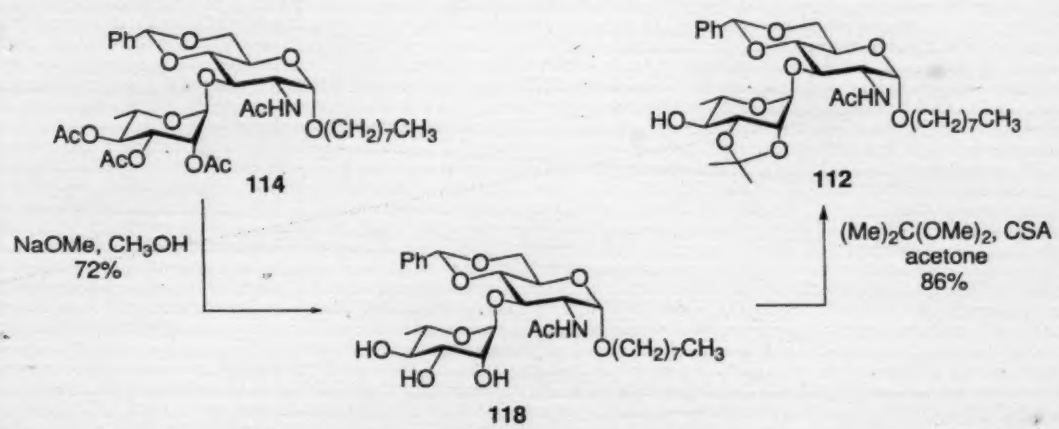
Scheme 50. Synthesis of disaccharide **9**.

Glycosylation of alcohol **111** with the peracetylated thioglycoside **113**[127] by activation with NIS and AgOTf afforded disaccharide **114** in 89% yield. The structure of the product was verified through its ^{1}H NMR spectrum, which showed the resonance for H-1 at 4.92 ppm as a doublet ($J_{1,2}$ = 3.7 Hz) and that for H-1' at 5.26 ppm as a doublet (H-1', $J_{1',2'}$ = 1.7 Hz); in the ^{13}C NMR spectrum, the two anomeric carbons appeared at 98.2 ppm (C-1') and 98.4 ppm (C-1). These NMR chemical shifts and coupling constants are characteristic of a disaccharide containing α-Glc*p*NAc and α-Rha*p* linkages. However, further support for the stereochemistry of the Rha*p* moiety in **114** was obtained by measurement of the $^{1}J_{C1\text{-}H1}$, which revealed a value of 170 Hz, consistent with the proposed α-stereochemistry.[174]

The protected disaccharide **114** was then treated with 80% aqueous acetic acid to remove the benzylidene acetal. The excess acetic acid was then evaporated and, without further purification, sodium methoxide in methanol was added to the crude product to remove all the acetyl protecting groups to give disaccharide **9** in 55% overall yield from **114**. The ^{1}H NMR spectrum of the final product showed the resonances at 4.88 ppm (H-1, $J_{1,2}$ = 3.6 Hz) and at 4.91 ppm (H-1', $J_{1,2}$ = 1.6 Hz) while the ^{13}C NMR spectrum showed the two anomeric carbons at 97.8 ppm and 102.1 ppm, supporting the proposed structure.

2.10.3 Synthesis of Oligosaccharides 10–12.

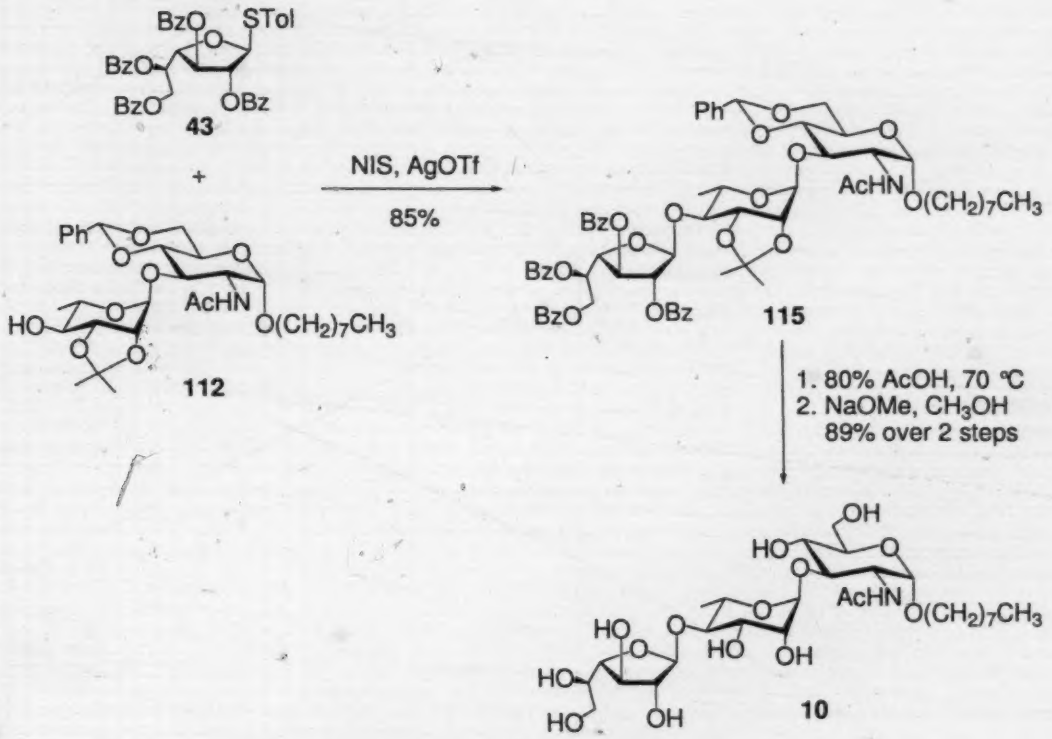
The preparation of compounds **10–12** was accomplished efficiently by utilizing a common disaccharide intermediate **112** as the acceptor in all glycosylation reactions. The synthesis of acceptor **112** from **114** is shown in Scheme 51. First, the acetyl protecting groups of **114** were removed under standard Zémplen conditions to give **118** in 72% yield. Subsequent protection of the hydroxyl groups at C-2 and C-3 as an



Scheme 51. Synthesis of acceptor **112**.

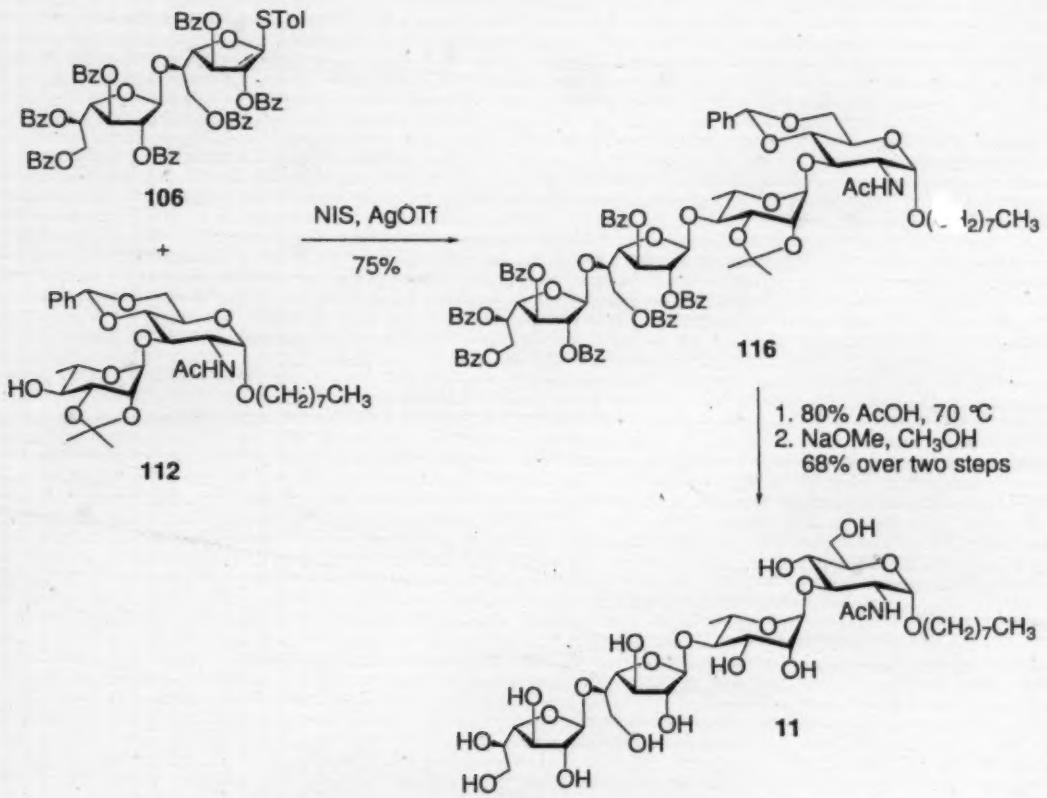
isopropylidene ketal provided **112** in 86% yield. The ^{1}H NMR spectrum of **112** showed the presence of two singlets at 1.55 and 1.32 ppm, which were attributed to the methyl groups of the isopropylidene ketal. In the ^{13}C NMR spectrum, a signal at 109.3 ppm was also present, which was due to the presence of the quaternary ketal carbon.

With **112** in hand, the assembly of **10–12** was patterned after the strategy for the synthesis of compounds **1–8** and involved NIS–AgOTf-promoted glycosylations of octyl glycoside acceptors with thioglycoside donors. Therefore, to synthesize **10**, the first step was coupling thioglycoside **43** with disaccharide **112** to give trisaccharide **115** in 85% yield (Scheme 52). Comparison of the ^{1}H NMR spectrum of **112** with that of **115** revealed an additional anomeric hydrogen signal, observed as a singlet at 5.78 ppm, which supports the incorporation of a β-Gal*f* residue into the molecule. Further support came from the presence of an anomeric carbon signal at 104.1 ppm in the ^{13}C NMR spectrum of **115**. Cleavage of benzylidene and isopropylidene protecting groups of intermediate **115** was achieved upon treatment with 80% acetic acid at 70 °C. Without further purification, this compound was debenzoylated under standard Zémplen conditions to yield trisaccharide **10** in 89% overall yield over two steps.



Scheme 52. Synthesis of Trisaccharide **10**.

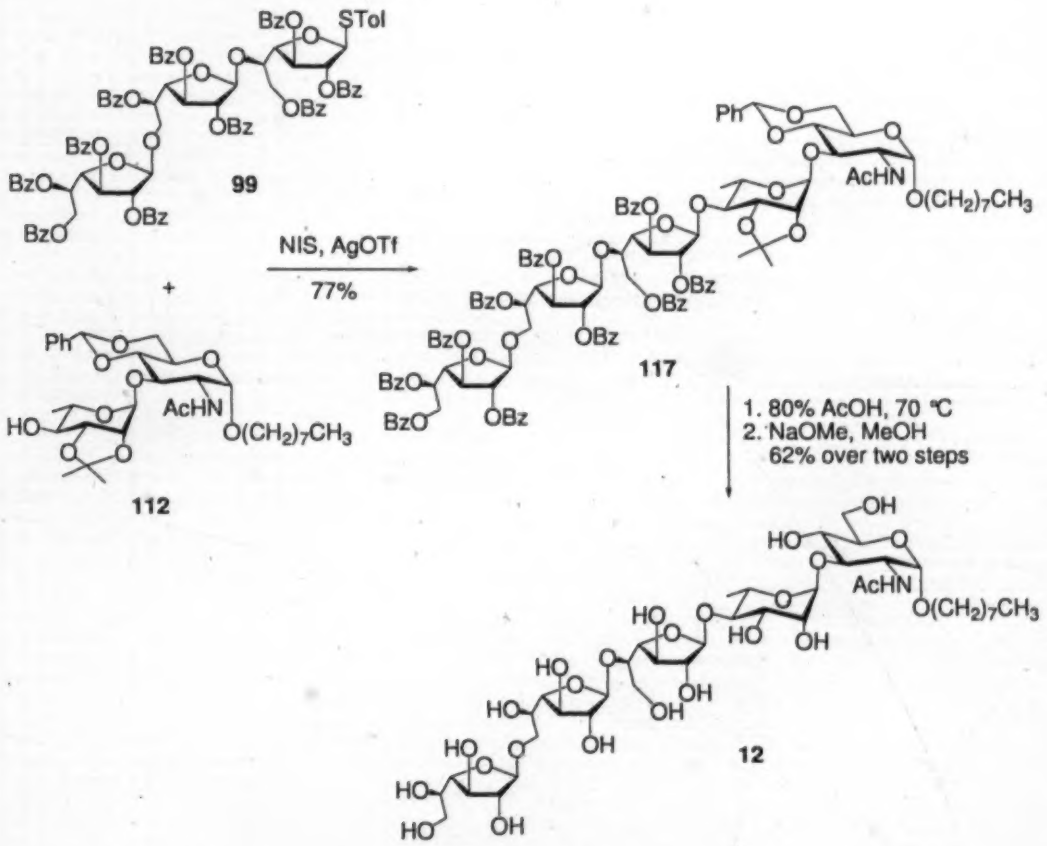
The synthesis of tetrasaccharide **11** is depicted in Scheme 53. Its preparation involved coupling of thioglycoside **106** with octyl glycoside acceptor **112** to afford tetrasaccharide **116** in 75% yield. Four anomeric carbon signals were present in the ^{13}C NMR spectrum of **116** at 105.5 (Gal*f*), 104.0 (Gal*f*), 98.2 (Glc*p*NAc) and 98.1 (Rha*p*) ppm, which are consistent with the proposed structure. Deprotection of this tetrasaccharide was accomplished as was done for **115** to yield **10**: cleavage of the acetals by treatment with 80% acetic acid at 70 °C followed by removal of the benzoyl protecting groups under basic conditions. Application of this sequence to **116** gave **11** in 68% overall yield over two steps.



Scheme 53. Synthesis of tetrasaccharide **11**.

Finally, the same general strategy was used to synthesize pentasaccharide **12** (Scheme 54). Disaccharide octyl glycoside **112** was glycosylated with thioglycoside donor **99** to give the protected pentasaccharide intermediate **117** in 62% yield. Deprotection of **117** was achieved in two steps as outlined above for the preparation of **10** and **11**, which gave **12** in 77% overall yield. In the ^{1}H NMR spectrum of **12**, the resonances at 5.26, 5.20 and 5.04 ppm were assigned to the anomeric hydrogens of the β-Gal*f* residues while those at 4.86 and 4.79 ppm arose from the α-Glc*p*NAc and α-Rha*p* residues, respectively. In the ^{13}C NMR spectrum, resonances for five anomeric carbons

were present at 109.3 (β-Gal*f*), 108.8 (β-Gal*f*), 108.1 (β-Gal*f*), 102.0 (α-Rha*p*), and 97.8 (α-Glc*p*NAc) ppm.



Scheme 54. Synthesis of pentasaccharide **12**.

2.11 Probing the Role of GlfT1 and GlfT2 in Galactan Biosynthesis with 9–12.

With oligosaccharides **9–12** synthesized, each was tested as a substrate for GlfT2. Dr. Natisha Rose, a research associate in the group, carried out this work. A full kinetic study was not done; each compound was simply subjected to the assay employed previously and the amount of radioactivity transferred to the product was measured. For

the sake of comparison, the best GftT2 substrate identified to date, trisaccharide **6**, was also included. The results are shown in Table 3.

Table 3. Ability of **9–12** to serve as acceptors for GlfT2[a]

Compound	dpm[b]
9	732
10	911
11	18,706
12	369,378
6	661,196
no acceptor	985

[a]Transfer of radiolabeled Gal*f* to each potential acceptor was measured using recombinant GlfB as described previously[76]. [b] Disintegrations per minute; average of duplicate runs.

As can be seen from the data presented in Table 3, neither disaccharide **9** or trisaccharide **10** are substrates for GlfT2 as the amount of transferred radioactivity is below background. On the other hand both, tetrasaccharide **11** and pentasaccharide **12** result in significant counts over background, indicating that they are substrates for this enzyme. These results indicate that our hypothesis about the role of GlfT2 in galactan assembly (Figure 25) was partially correct in that the enzyme does not add the first and second Gal*f* residues. However, unlike our initial hypothesis, it appears that GlfT2 add the third, fourth, and remaining Gal*f* residues. These results were confirmed by additional work carried out by our collaborators Professors Katarina Mikusova (Comenius University, Bratislava, Slovakia) and Patrick J. Brennan (Colorado State University) and their research groups. Glycolipids **118** and **119** (Figure 26), which correspond to early intermediates in galactan biosynthesis were isolated from

mycobacteria and then incubated with GlfT2 and UDP-Gal*f*. The use of these naturally-occurring analogs of **11** and **12** in this assay led to a full-length galactan structure, thus supporting our hypothesis that GlfT2 is responsible for the addition of the third and all subsequent Gal*f* residues in mycobacterial galactan.[84]

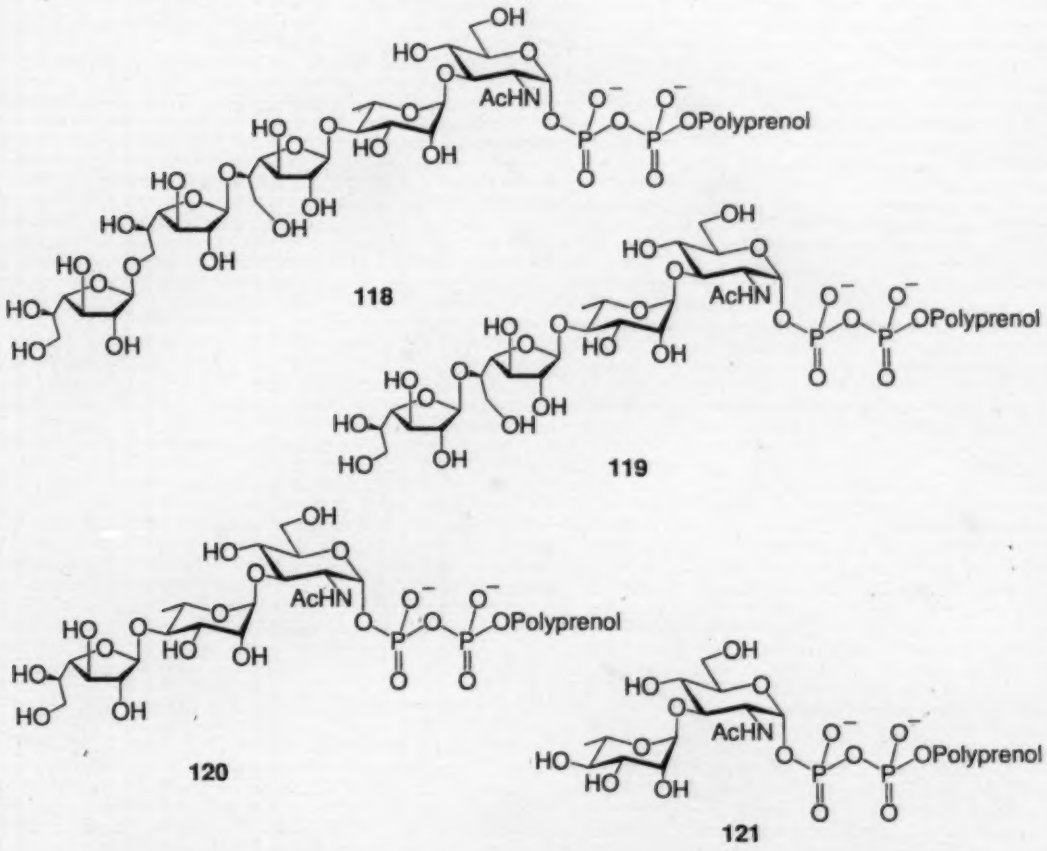


Figure 26. Galactan biosynthetic precursors isolated from mycobacteria.

A question left outstanding by the studies described above is the nature of the enzymes that add the first two Gal*f* residues to the polymer. About the same time we published our initial studies on GlfT2,[76] a report appeared suggesting that mycobacteria

produce a second, bifunctional galactofuranosyltransferase (GlfT1), which is encoded by the Rv3782 gene.[82] It was further proposed that GlfT1 is responsible for the incorporation of the first two Gal*f* residues into mycobacterial galactan. Professors Mikusova and Brennan and their groups used **9** and **10**, as well as their naturally occurring analogues **120** and **121** (Figure 26), to establish the bifunctionality of this enzyme. As outlined in Figure 27, incubation of **9** with GlfT1 and UDP-Gal*f* led to production of **10**; a similar experiment with **10** led to **11**. However, when **11** was used as the substrate, no product was formed. These studies clearly demonstrate that GlfT1 adds the first two Gal*f*

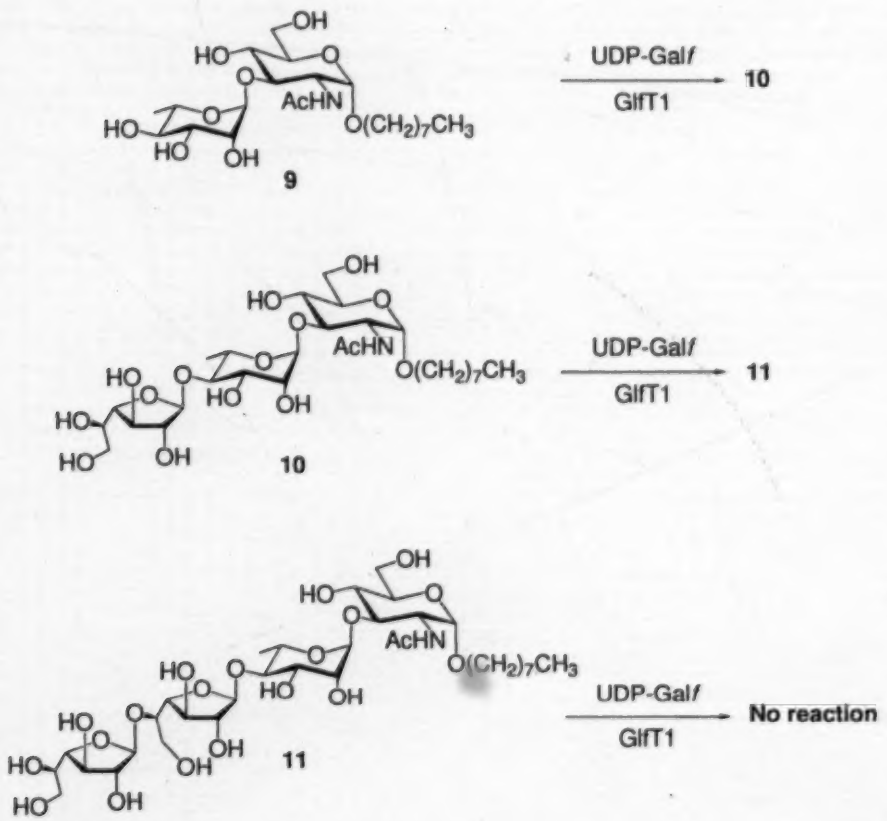


Figure 27. Results of incubating **9–12** with UDP-Galf in the presence of GlfT1.[84]

residues, but not the third. When the same experiments were done with disaccharide glycolipid **120** the only product detected was the one resulting from two Gal*f* additions (**119**); the same product resulted when **121** was used.

These latter results reveal a difference in reactivity between the naturally occurring (**120** and **121**) and synthetic octyl glycosyide (**9** and **10**) substrates. While GlfT1 will add two Gal*f* residues to **120**, it will transfer only one monosaccharide to its octyl glycoside analog, **9**. While we do not understand if the origin of this difference is specificity, clearly the identity of the lipid and its linkage to the carbohydrate influences the enzymatic glycosylation. However, it should be noted that this subtle discriminatory effect can be overridden by using a substrate containing the first Gal*f* residue. Both **10** and **121** are GlfT1 substrates that lead to a product containing two Gal*f* residues.

Taken together the use of compounds **9–12** by us and our collaborators, have allowed us to develop a clearer picture of the process by which the galactan is assembled (Figure 28). The picture that emerges is that only two bifunctional galactofuranosyl

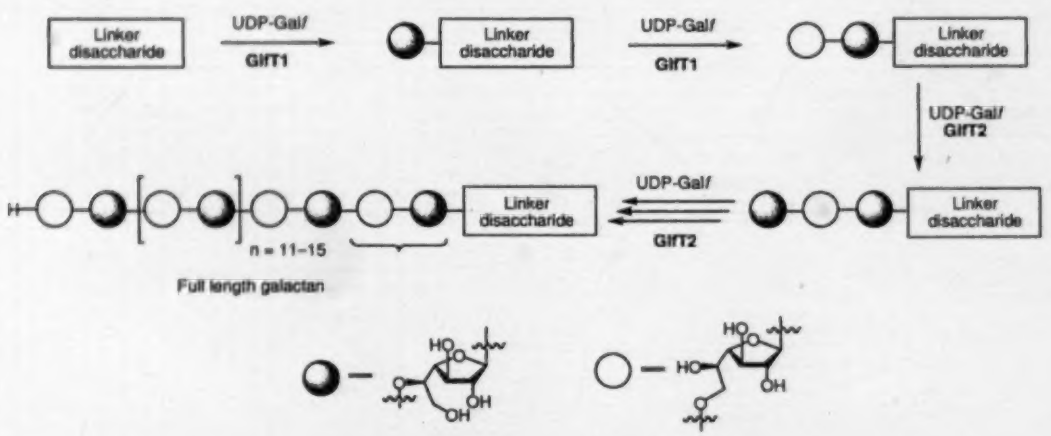


Figure 28. Steps involved in the biosynthesis of mycobacterial galactan.[84]

transferases, GlfT1 and GlfT2, are responsible for the assembly of the galactan chain. The glycosyltransferases that add the Rha*p* and Glc*p*NAc residues have also been identified (but not well characterized)[38] and therefore all of the steps in the synthesis of the arabinogalactan core have been established.

2.12 Conclusions

In conclusion, we have successfully synthesized a panel of Gal*f*-containing oligosaccharides (**1–12**) that were used as probes for the specificity of two galactofuranosyltransferases, GlfT1 and GlfT2, which are involved in mycobacterial galactan biosynthesis. In the synthesis of the compounds, we exploited an iodine-promoted dithioacetal cyclization to generate the required Gal*f* building blocks. This is the first use of this reaction in the synthesis of Gal*f*-containing glycoconjugates and advantages of this approach include simplicity, an inexpensive and relatively non-toxic promotor, and the ability to generate furanosides without contamination by pyranoside isomers.

With all of the appropriate building blocks in place, the assembly of the oligosaccharides was carried out largely by the use of NIS–AgOTf-promoted glycosylations of thioglycoside donors and octyl glycoside acceptors. In some instances, however, trichloroacetimidate donors were also employed. The general strategy applied to the synthesis of the compounds was to add the monosaccharides from the reducing to non-reducing end but occasionally the opposite approach was employed. In particular, this was necessary to prepare one of the tetrasaccharide targets, **8**. Its preparation was only successful by an approach in which the molecule was assembled from the non-reducing to reducing end.

Another achievement was the development of an efficient one-pot strategy for the synthesis of two key trisaccharides, **5** and **6**. As large amounts of these materials are needed as substrates for characterization of GlfT2 and identification of inhibitors of this

enzyme, it was necessary to develop a robust approach for accessing multi-milligram quantities of these oligosaccharides.

We have also completed, for the first time, the synthesis of oligosaccharides **9–12**, which contain the α-Rha*p*-(1→3)-α-Glc*p*NAc linker disaccharide that connects that arabinogalactan to the peptidoglycan in the mycobacterial cell wall. The strategy in assembling these compounds was to react a protected derivative of α-Rha*p*-(1→3)-α-Glc*p*NAc-O$(CH_2)_7CH_3$, with a thioglycoside containing 1–3 Gal*f* residues.

Oligosaccharides **9–12** were used in collaborations with Dr. Natisha Rose in our laboratory as well as Professors Katarina Mikusova and Patrick Brennan, to establish that only two bifunctional galactofuranosyltransferases GlfT1 and GlfT2, are involved in the assembly of mycobacterial galactan. In conjunction with Dr. Rose, oligosaccharides **1–8** were use to characterize the substrate specificity of GlfT2. In these latter investigations, the bifunctionality of the enzyme was established and the best substrate reported, to date, for the enzyme (trisaccharide **5**) was identified.

In sum, the work described in this thesis has led to the development of new methodologies for the assembly of oligosaccharides containing Gal*f* residues and their application to the synthesis of 12 target oligosaccharides, which are fragments of the mycobacterial cell wall polysaccharide, arabinogalactan. Furthermore, in collaborations with individuals in our laboratory, and others, these compounds have been used to probe the biosynthesis of arabinogalactan and the specificity of the two key enzymes in the assembly of the galactan portion of the polysaccharide.

2.13 Future Work

Evidence presented in this work and with our collaborators showed the involvement of two bifunctional galactocytofuranosyltransferases during biosynthesis of the entire galactan chain in mycobacterial AG complex. As a possible extension of this research, it is proposed that compounds **122-127** be synthesized and tested as substrates or potential competitive inhibitors of GlfT1 (Figure 29).

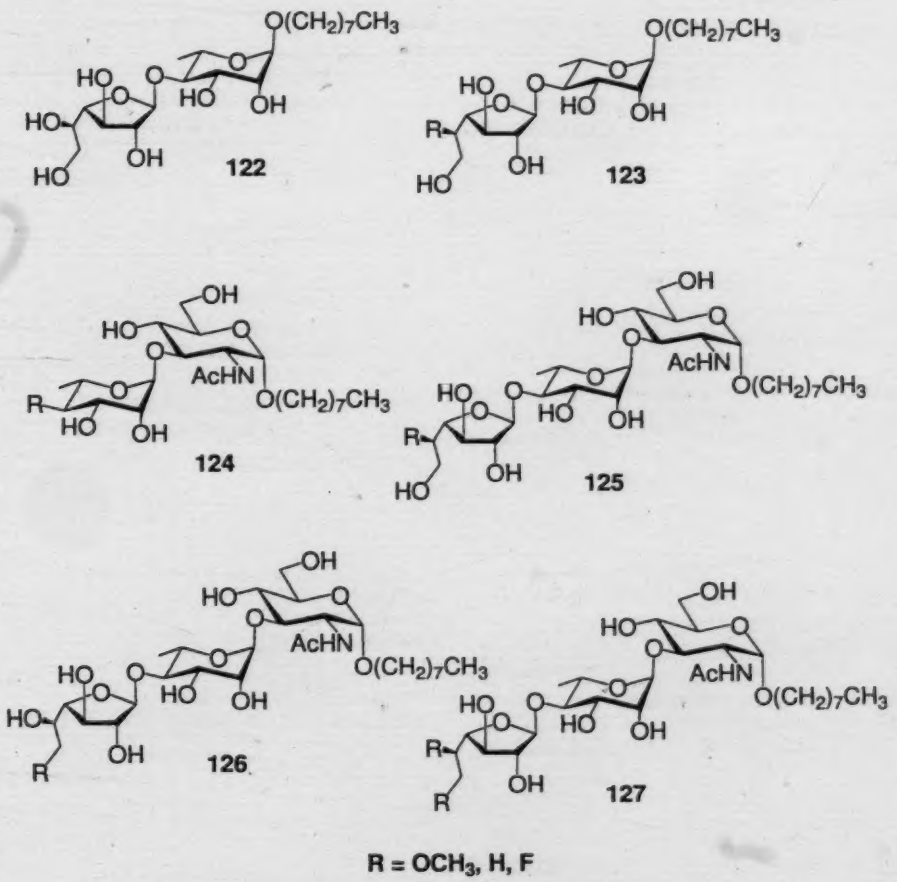


Figure 29. Potential substrates and/or inhibitors of GlfT1.

It would be interesting to know if the Glc*p*NAc moiety is necessary for the catalytic action of GlfT1 (i.e. compound **122**), or whether GlfT1 is also able to recognize substrate such as compounds **123** and **126**. It was established in our collaborative work that GlfT1 is able to catalyze the sequential formation of a (1→4) and (1→5) linkages in a growing substrate during glycosylation reaction. Noting that its second catalytic action is the formation of a (1→5) linkage during such reaction, it would be worthwhile to investigate whether GlfT1 is able to recognize a substrate analog (i.e. compound **123**) having a hydroxymethyl group at C-6" position even though the OH group in the C-5" position is not available. Conversely, will an analog (e.g. compound **126**) having no OH group in the C-6'' position be also considered by the enzyme as a substrate? This experiment should serve to illustrate whether perturbations in the position(s) adjacent to the donor's glycosylation site will help in the development of more effective potential inhibitors.

Likewise, potential inhibitors of GlfT2 such as those shown in Figure 30 may also be prepared. A similar one-pot synthesis, as described for compounds **5** and **6**, may be used to prepare these potential inhibitors (Figure 31). This can be achieved by preparing a disaccharide/trisaccharide imidate donor, followed by coupling with the appropriate thioglycoside donor. Once the new thioglycoside intermediate is formed, the disaccharide octyl acceptor can be added to form the desired product. This synthetic strategy should enable us to expeditiously prepare potential inhibitors and the necessary analogs for testing on the desired target mycobacterial enzymes.

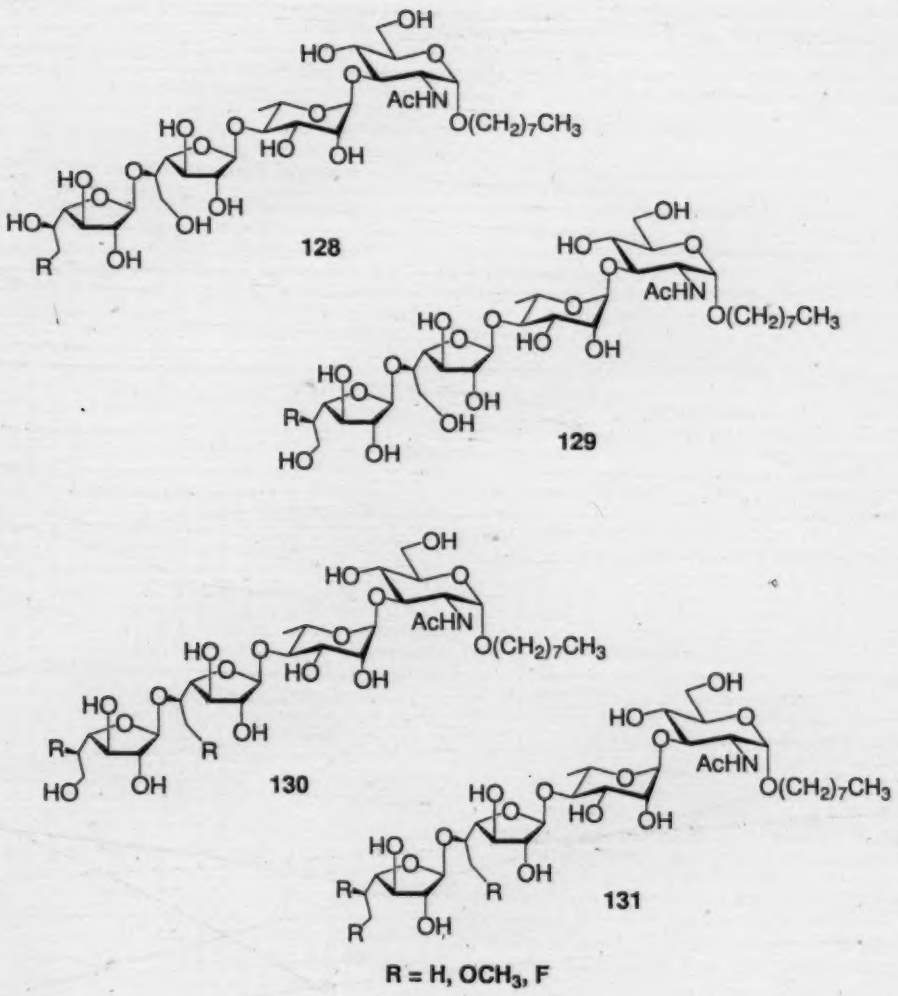


Figure 30. Potential inhibitors of GlfT2.

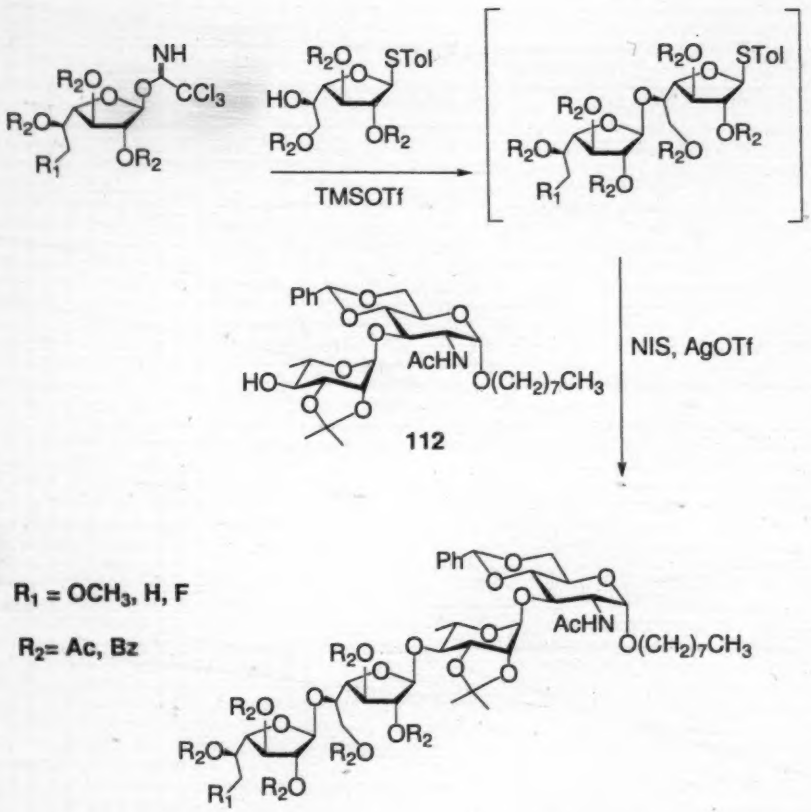


Figure 31. Proposed one-pot synthesis of potential inhibitor of GlfT2.

Figure 32 shows additional potential inhibitors of GlfT2 having conformationally restricted core structures. The proposed compounds contain 2,3-anhydrosugar moiety which is expected to result to a marked increase in their lipophilic property and bioavailability. Moreover, the presence of the epoxide moiety is expected to lock the galactofuranose ring in a low energy conformation (E_O) (Figure 33), which is similar to the conformation adopted by β-galactofuranosides.[M. Richards, personal communication]

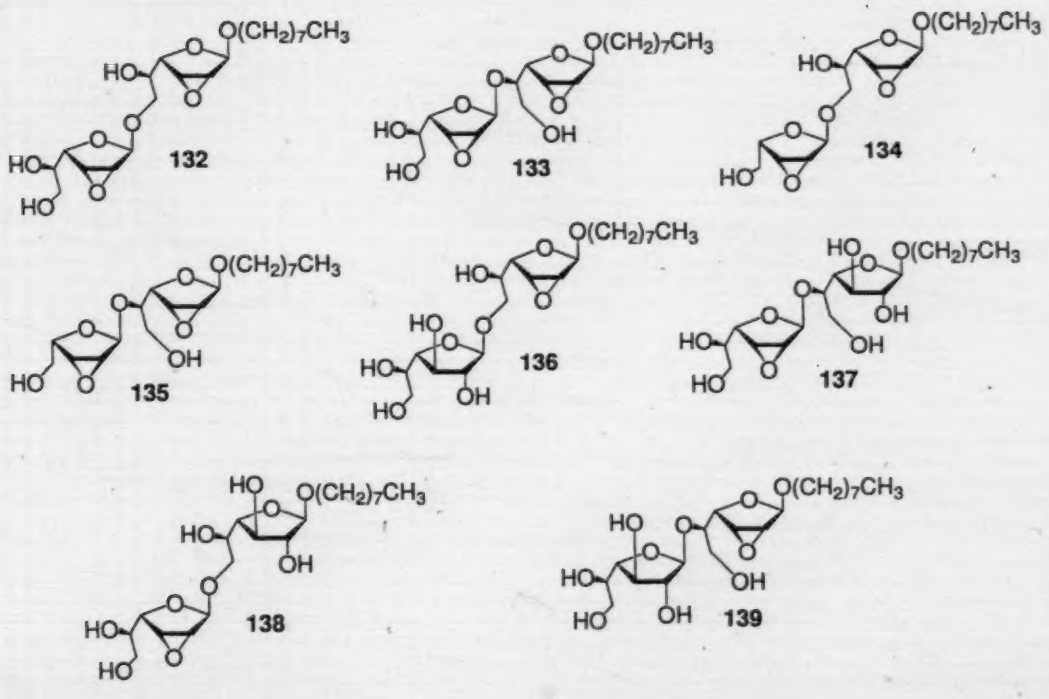


Figure 32. Potential inhibitors of GlfT2 with conformationally restricted core structures.

Figure 33. Minimum energy-optimized structure of galactofuranose ring containing 2,3 anhydrosugar residue using MM2 (left panel).

From the enzymes' bifunctional nature, it can be rationalized that there may be two active sites or two transferase activities in GlfT1 and GlfT2. Therefore, a logical progression of this research is to obtain crystal structures of GlfT1 and GlfT2, which as of yet, have not been done. Our laboratory has a collaboration with Prof. Kenneth Ng (University of Calgary) to obtain a crystal structure of GlfT2. A crystal structure showing substrate-enzyme complex, inhibitor-enzyme complex and/or donor/acceptor-enzyme complex will be important to further understand the enzyme's catalytic mechanism of action. This information can then be used to design better inhibitors of these enzymes.

Moreover, different analogs of UDP-Gal*f* can also be synthesized chemo-enzymatically for use as potential inhibitors and biochemical probes to provide insight into the structural features of the enzyme's donor binding site (Figure 34). Systematic replacement of the functional groups at C-2, C-3, C-4 and C-5 positions will be useful in probing the binding site of the donor. This can be achieved by observing the effects of functional group replacement, with regards to steric effects, hydrogen bonding, electrostatic interaction and other noncovalent interactions, to the activity of the enzyme.

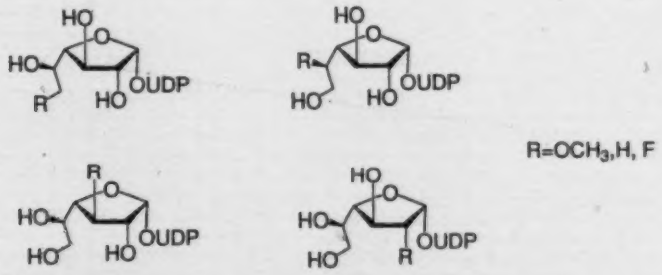
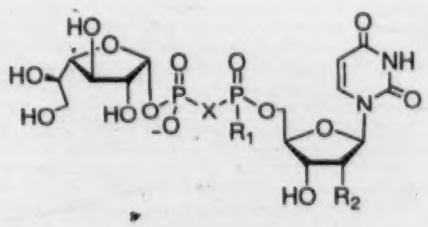


Figure 34. Proposed donor analogs of UDP-Gal*f*.

Another approach that can be taken is to perform bioisosteric replacement[175] of the key functional groups, such as those marked with X or R, in the phosphate substrates to determine whether this will result to a marked improvement in the physico-chemical and biological properties of a potential inhibitor (Figure 35). In this particular example, an obvious bioisosteric replacement is that of the phosphate group with either a sulfate or carboxylate group.[175,176] Another bioisosteric interchange that can be done is that of an amino group for the hydroxyl moiety. These two functional groups are often used as bioisosteres due to its similarity in steric size, spatial arrangement and their hydrogen binding capability.[175] These factors are very important since the proposed experiment involved binding between a substrate and an enzyme. A systematic substitution of an amino for a hydroxyl group in one or more positions in the proposed inhibitor(s) should facilitate tuning the binding properties of the putative inhibitor to the enzyme. Once a potential inhibitor or lead compound is determined, this experiment can be expanded to a detailed quantitative structure activity relationship (QSAR) studies to help establish a better understanding of the pharmacophoric sites of the enzyme of interest and, perhaps, could lead to drug discovery.[175] This approach may also be applied to the other proposed inhibitors mentioned above.



X = O, CF_2, CH_2; R_1 = BH_3; R_2 = H, N_3

Figure 35. Potential phosphate analogs.

STD NMR and molecular modeling studies can also be done to elucidate the binding nature of UDP-Gal*f* with GlfT2 and binding of the acceptor substrates with GlfT2. Acceptor substrates **5** and **6**, being better substrates than disaccharides **1** and **2**, can be used in these studies. To better understand and identify epitopes in the substrates and donor (UDP-Gal*f*) that influence binding to GlfT2, several compounds (Figure 36) can be synthesized and used as probes to provide insight into the structural features of the substrate binding site and compounds in Figure 34 for the donor binding site. This project is on-going in collaboration with Prof. B. Mario Pinto (Simon Fraser University) and initial investigation has been done to study the binding of GlfT2 with acceptor substrates **5** and **6** and native UDP-Gal*f* donor.

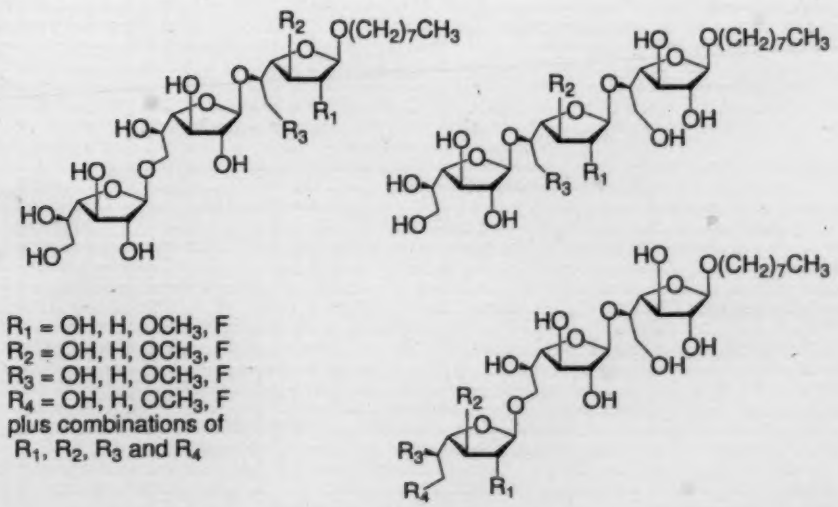


Figure 36. Potential substrates to probe binding of the acceptor with GlfT2.

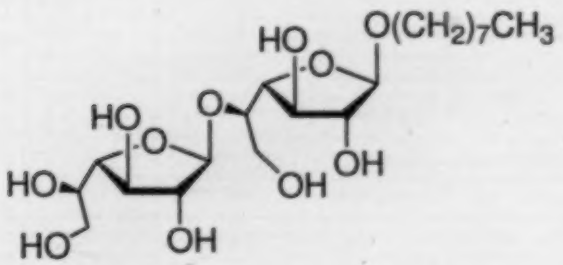
Another method that can be used to probe the enzyme's active site is to perform site-directed mutagenesis studies on the conserved DxD motif residues. Results on these studies would give an insight on which residues are involved in the active site and catalysis of the enzyme. GlfT2, a member of the GT-2 glycosyltransferase family in *M. tuberculosis*, is generally characterized to carry a DxD motif found to bind a divalent cation as part of immobilization of the sugar-nucleotide donor.[177] A crystal structure of SpsA, an GT-2 enzyme involved in spore formation of *Bacillus subtilis*, showed that at least three invariant residues, Asp^{39}, Asp^{98}, and Asp^{99} in the N-terminal domain are involved in binding of the donor substrate.[38] Mutations on conserved Asp residues in GlfT2 (Asp^{39}, Asp^{97}, and Asp^{99}) to either glutamate, asparagine or lysine might give us an idea of which residues are involved in binding of the donor substrate, UDP-Gal*f*.

CHAPTER 3

EXPERIMENTAL PROCEDURES

3.1 General Methods. Reactions were carried out in oven-dried glassware. All reagents were purchased from commercial sources and were used without further purification unless noted. Reaction solvents were purified by successive passage through columns of alumina and copper under nitrogen. Unless stated otherwise, all reactions were carried out at room temperature under a positive pressure of argon and were monitored by TLC on Silica Gel 60 F_{254} (0.25 mm, E. Merck). TLC spots were detected under UV light or by charring with acidified *p*-anisaldehyde solution in EtOH. All organic solutions were dried with anhydrous $MgSO_4$. Unless otherwise indicated, all column chromatography was performed on Silica Gel (40–60 μM). The ratio between silica gel and crude product ranged from 100 to 50:1 (w/w). Optical rotations were measured at 22 ± 2 °C. ^{1}H NMR spectra were recorded at 600 MHz, 500 MHz or 400 MHz, and chemical shifts were referenced to either TMS (0.0 ppm, $CDCl_3$), CD_3OD (3.30 ppm, CD_3OD) or HOD (4.78 ppm, D_2O). ^{1}H data were reported as though they were first order. ^{13}C NMR (APT) spectra were recorded at 125 MHz or 100 MHz, and ^{13}C chemical shifts were referenced to internal $CDCl_3$ (77.23 ppm, $CDCl_3$), or CD_3OD (48.9 ppm, CD_3OD) or external acetone (31.07, D_2O). Assignments of resonances in NMR spectra were made using ^{1}H–^{1}H COSY and HMQC experiments. Organic solutions were concentrated under vacuum at < 40 °C. Electrospray mass spectra were recorded on samples suspended in mixtures of THF with MeOH and added NaCl. MALDI mass spectrometry was performed on a Voyager Elite time-of-flight spectrometer on samples suspended in 2,5-dihydroxy benzoic acid, IAA (*trans*-3-indoleacrylic acid) or DCTB (2-[(2*E*)-3-(4-*tert*-butylphenyl)-2-methylprop-2-enylidene]-malononitrile) using the delayed-extraction mode and positive-ion detection.

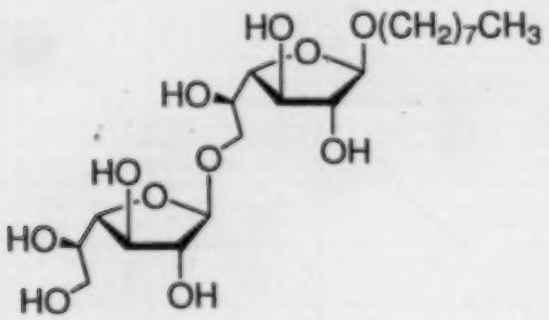
3.2 General Procedure for the Deprotection of Perbenzoylated Intermediates Leading to Compounds 1-8. The starting material was dissolved in 3:1 MeOH–CH_2Cl_2 (10–50 mL) followed by dropwise addition of NaOMe in MeOH (0.1 M) until the pH of the solution was 12. The reaction mixture was stirred for 4 h and was neutralized by the careful addition of Amberlyst-15 (H^+) cation exchange resin. The solution was filtered and the filtrate concentrated to give a syrupy residue. The resulting crude product was then purified by column chromatography. The solvent was evaporated and the residue was redissolved in water before filtration through a Sep-pak C-18 cartridge.



1

Octyl β-D-galactofuranosyl-(1→5)-β-D-galactofuranoside (1). Using the general deprotection procedure, octyl glycoside **77** (92.0 mg, 0.078 mmol) gave a crude product which was purified by column chromatography (5:1 CH_2Cl_2–MeOH). The solvent was evaporated and the residue was redissolved in water before filtration through a Sep-pak C-18 cartridge to afford **1** as a clear, colorless oil (32.0 mg, 90%). R_f 0.31 (7:1 CH_2Cl_2–MeOH,); $[\alpha]_D$ –90.0 (*c* 1.0, MeOH); ^{1}H NMR (500 MHz, CD_3OD, δ_H) 5.19 (br s, 1 H, H-1'), 4.84 (br s, 1 H, H-1), 4.14 (dd, 1 H, *J* = 5.5, 3.0 Hz, H-3'), 4.07 (dd, 1 H, *J* = 6.8, 3.9 Hz, H-3), 4.04 (dd, 1 H, *J* = 3.0, 1.5 Hz, H-2'), 4.02–3.98 (m, 1 H, H-4'), 3.94 (dd, 1 H, *J*

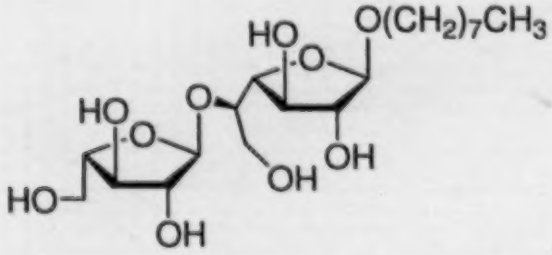
= 3.9, 1.9 Hz, H-2), 3.90–3.80 (m, 1 H, H-4), 3.75–3.72 (m, 3 H, H-5, H-6_a, H-6_b), 3.68–3.45 (m, 5 H, H-5', H-6_a', H-6_b', octyl OCH_2), 1.60–1.55 (m, 2 H, octyl CH_2), 1.37–1.26 (m, 10 H, octyl CH_2), 1.20–1.00 (m, 3 H, octyl CH_3); ^{13}C NMR (125 MHz, CD_3OD, δ_C) 110.1 (C-1'), 109.3 (C-1), 85.1 (C-2'), 84.7 (C-2), 83.5 (C-4'), 82.9 (C-4), 78.9 (C-3), 78.9 (C-3'), 72.5 (C-5), 71.0 (C-5'), 70.7 (C-6), 68.9 (octyl OCH_2), 64.5 (C-6'), 33.0 (octyl CH_2), 33.0 (octyl CH_2), 33.0 (octyl CH_2), 30.7 (octyl CH_2), 30.7 (octyl CH_2), 27.2 (octyl CH_2), 14.5 (octyl CH_3). ESI-MS *m/z* calcd. for (M + Na) $C_{20}H_{38}O_{11}Na$: 477.2306. Found: 477.2306.



2

Octyl β-D-galactofuranosyl-(1→6)-β-D-galactofuranoside (2). Using the general deprotection procedure, octyl glycoside **78** (37.1 mg, 0.032 mmol) gave a crude product, which was purified by column chromatography (5:1 CH_2Cl_2–MeOH). The solvent was evaporated and the residue was redissolved in water before filtration through a Sep-pak C-18 cartridge to furnish **2** as a colorless oil (12.5 mg, 87%). R_f 0.31 (7:1 CH_2Cl_2–MeOH,); $[\alpha]_D$ –85.0 (*c* 1.0, MeOH); ^{1}H NMR (500 MHz, CD_3OD, δ_H) 4.91 (d, 1 H, *J* = 1.2 Hz, H-1'), 4.83 (d, 1 H, *J* = 1.8 Hz, H-1), 4.20–3.94 (m, 4 H, H-3', H-3, H-2', H-4'), 3.92 (dd, 1 H, *J* = 3.8, 1.8 Hz, H-2), 3.88–3.84 (m, 2 H, H-4, H-6_a), 3.82 (dd, 1 H, *J* =

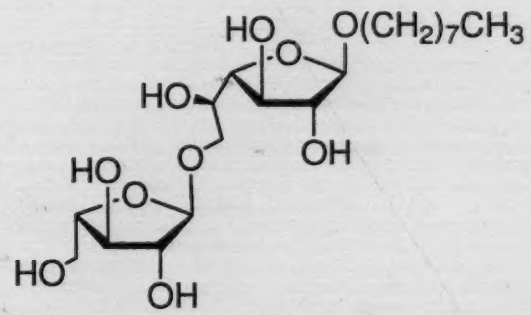
10.0, 4.0 Hz, H-6_a'), 3.74–3.66 (m, 2 H, H-5', octyl OCH_2), 3.64–3.58 (m, 2 H, H-6_b', H-5), 3.54 (dd, 1 H, J = 10.0, 6.8 Hz, H-6_b), (ddd, 1 H, J = 9.5, 7.0, 7.0 Hz, octyl OCH_2), 1.60–1.37 (m, 2 H, octyl CH_2), 1.37–1.25 (m, 10 H, octyl CH_2), 0.88 (t, 3 H, J = 7.0 Hz, octyl CH_3); ^{13}C NMR (125 MHz, CD_3OD, δ_C) 110.0 (C-1'), 109.3 (C-1), 85.1 (C-2'), 84.7 (C-2), 83.5 (C-4'), 82.9 (C-4), 78.9 (C-3, C-3'), 72.5 (C-5), 71.0 (C-5'), 70.7 (C-6'), 68.9 (octyl OCH_2), 64.5 (C-6), 33.03 (octyl CH_2), 33.0 (octyl CH_2), 30.0 (octyl CH_2), 30.74 (octyl CH_2), 30.7 (octyl CH_2), 27.2 octyl (CH_2), 14.5 (octyl CH_3). ESI-MS m/z calcd. for (M + Na) $C_{20}H_{38}O_{11}Na$: 477.2306. Found: 477.2306.



3

Octyl β-L-arabinofuranosyl-(1→5)-β-D-galactofuranoside (3). Using the general deprotection procedure, octyl glycoside **79** (53.0 mg, 0.05 mmol) gave a crude product, which was purified by column chromatography (6:1 CH_2Cl_2–MeOH). The solvent was evaporated and the residue was redissolved in water before filtration through a Sep-pak C-18 cartridge to afford **3** as clear, colorless oil (19.3 mg, 90%). R_f 0.31 (4:1 CH_2Cl_2–MeOH,); $[\alpha]_D$ –95.0 (c 1.0, MeOH); ^{1}H NMR (500 MHz, CD_3OD, δ_H) 5.19 (d, 1 H, J = 0.5 Hz, H-1'), 4.82 (d, 1 H, J = 1.8 Hz, H-1), 4.10 (dd, 1 H, J = 8.7, 5.0 Hz, H-4'), 4.04 (dd, 1 H, J = 6.5, 3.0 Hz, H-3), 4.01 (dd, 1 H, J = 3.0, 0.5 Hz, H-2'), 3.98 (dd, 1 H, J = 6.5, 4.1 Hz, H-4), 3.92–3.89 (m, 3 H, H-6_a, H-2, H-5), 3.94 (d, 1 H, J = 5.0, 3.0 Hz, H-

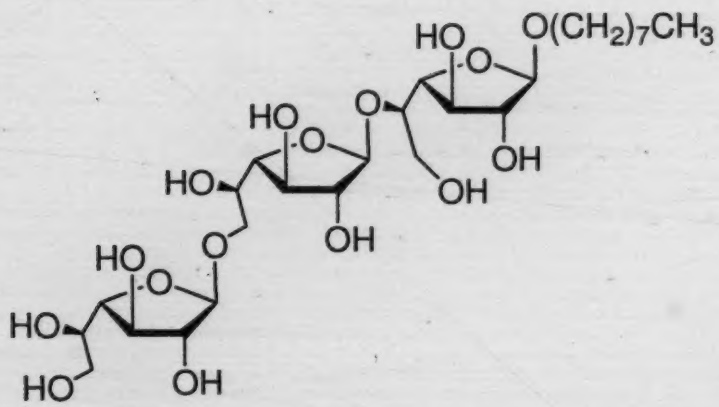
3'), 3.88–3.77 (m, 4 H, H-6_b, H-5_a', octyl OCH_2, H-5_b'), 3.54 (ddd, 1 H, J = 9.5, 7.0, 7.0 Hz, octyl OCH_2), 1.60–1.37 (m, 2 H, octyl CH_2), 1.37–1.25 (m, 10 H, octyl CH_2), 0.88 (t, 3 H, J = 7.0 Hz, octyl CH_3); ^{13}C NMR (125 MHz,CD_3OD δ_C) 109.4 (C-1'), 109.1 (C-1), 86.7 (C-4'), 83.8 (C-4), 83.7 (C-2), 82.5 (C-2'), 78.9 (C-3), 78.8 (C-3'), 77.2 (C-5), 68.9 (octyl OCH_2), 63.3 (C-5'), 62.7 (C-6), 33.0 (3 x octyl CH_2), 30.72 (octyl CH_2), 30.70 (octyl CH_2), 27.3 (octyl CH_2), 14.4 (octyl CH_3). ESI-MS *m/z* calcd. for (M + Na) $C_{19}H_{36}O_{10}Na$: 447.2206. Found: 447.4831.



4

Octyl β-L-arabinofuranosyl-(1→6)-β-D-galactofuranoside (4). Using the general deprotection procedure, octyl glycoside **80** (80.6 mg, 0.077 mmol) gave a crude product, which was purified by column chromatography (6:1 CH_2Cl_2–MeOH). The solvent was evaporated and the residue was redissolved in water before filtration through a Sep-pak C-18 cartridge to afford **4** as a clear, colorless oil (30.0 mg, 92%). R_f 0.31 (4:1 CH_2Cl_2–MeOH,); $[\alpha]_D$ –90.0 (*c* 1.0, MeOH); ^{1}H NMR (500 MHz, CD_3OD, δ_H) 4.91 (s, 1 H, H-1'), 4.82 (d, 1 H, J = 1.5 Hz, H-1), 3.99–3.95 (m, 3 H, H-3, H-2', H-5), 3.94 (d, 1 H, J = 1.5 Hz, H-2), 3.88–3.77 (m, 4 H, H-4, H-4', H-3', H-5_a'), 3.78–3.64 (m, 3 H, H-6_a, octyl OCH_2, H-6_b), 3.54 (dd, 1 H, J = 11.0, 3.0 Hz, H-5_b'), 3.40 (ddd, 1 H, J = 9.5, 7.0, 7.0 Hz, octyl OCH_2), 1.60–1.37 (m, 2 H, octyl CH_2), 1.37–1.25 (m, 10 H, octyl CH_2), 0.88 (t, 3

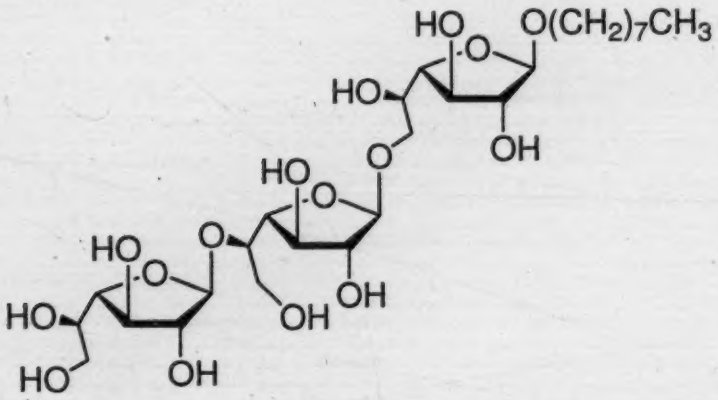
H, J = 7.0 Hz, octyl CH_3); ^{13}C NMR (125 MHz, CD_3OD, δ_C), 110.1 (C-1'), 109.3 (C-1), 86.0 (C-2'), 84.6 (C-2), 83.6 (C-4'), 83.2 (C-4), 78.8 (C-3'), 78.8 (C-3), 71.0 (C-5), 70.6 (C-5'), 68.9 (octyl OCH_2), 63.1 (C-6), 33.0 (octyl CH_2), 30.7 (octyl CH_2), 30.6 (octyl CH_2), 30.4 (octyl CH_2), 27.3 (octyl CH_2), 23.7 (octyl CH_2), 14.5 (octyl CH_3). ESI-MS m/z calcd. for (M + Na) $C_{19}H_{36}O_{10}Na$: 447.2206. Found: 447.4839.



5

Octyl β-D-galactofuranosyl-(1→6)-β-D-galactofuranosyl-(1→5)-β-D-galactofuranoside (5). Using the general deprotection procedure, octyl glycoside **85** (45.4 mg, 0.027 mmol) gave a crude product, which was purified by column chromatography (3:1 CH_2Cl_2–MeOH). The solvent was evaporated and the residue was redissolved in water before filtration through a Sep-pak C-18 cartridge to furnish trisaccharide **3** as a clear, colorless oil (15.2 mg, 90%). R_f 0.21 (3:1 CH_2Cl_2–MeOH); $[\alpha]_D$ –93.0 (c 1.0, MeOH); ^{1}H NMR (500 MHz, D_2O, δ_H): 5.23 (d, 1 H, J = 1.3 Hz, H-1''), 5.04 (d, 1 H, J = 1.5, H-1'), 4.98 (d, 1 H, J = 2.0 Hz, H-1), 4.14–3.94 (m, 9 H, H-2'', H-2', H-3', H-2, H-3, H-4, H-4', H-3'', H-4''), 3.90 (dd, 1 H, J = 7.0, 3.5 Hz, H-6_a'), 3.61–3.75 (m, 3 H, H-5', H-5'', H-6_b'), 3.74–3.61 (m, 6 H, H-5, H-6_a, octyl OCH_2, H-

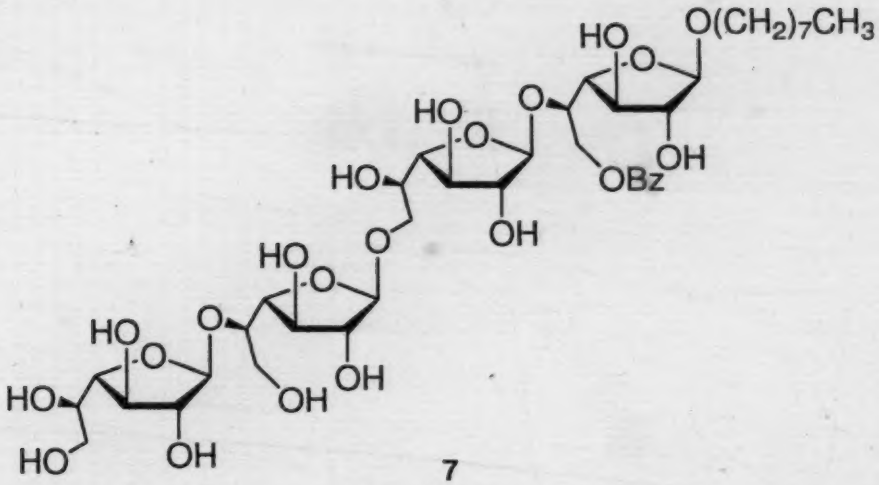
6_b'', H-6_a'', H-6_b), 3.54 (ddd, 1 H, J = 10.0, 7.5, 7.5 Hz, octyl OCH_2), 1.60–1.37 (m, 2 H, octyl CH_2), 1.37–1.25 (m, 10 H, octyl CH_2), 0.88 (t, 3 H, J = 7.0 Hz, octyl CH_3); ^{13}C NMR (125 MHz, D_2O, δ_C): 108.7 (C-1'), 108.0 (C-1), 107.9 (C-1''), 83.9 (C-4''), 83.8 (C-4), 82.3 (C-4'), 82.1 (C-2'), 81.8 (C-2), 81.2 (C-2''), 77.7 (C-3'), 77.6 (C-3''), 77.3 (C-3), 76.8 (C-5), 71.7 (C-5'), 70.4 (C-5''), 70.2 (C-6'), 69.6 (octyl OCH_2), 63.8 (C-6), 61.9 (C-6''), 32.0 (octyl CH_2), 29.5 (octyl CH_2), 29.3 (octyl CH_2), 29.2 (octyl CH_2), 26.1 (octyl CH_2), 22.9 (octyl CH_2), 14.5 (octyl CH_3). ESI-MS m/z calcd. for $[M + Na]^+$ $C_{26}H_{48}O_{16}Na$: 639.2837. Found: 639.2835.



6

Octyl β-D-galactofuranosyl-(1→5)-β-D-galactofuranosyl-(1→6)-β-D-galactofuranoside (6). Using the general deprotection procedure, octyl glycoside **88** (60.3 mg, 0.036 mmol) gave a crude product, which was purified by column chromatography (3:1 CH_2Cl_2–MeOH). The solvent was evaporated and the residue was redissolved in water before filtration through a Sep-pak C-18 cartridge to furnish **6** as a clear, colorless oil (20.0 mg, 89%). R_f 0.24 (3:1 CH_2Cl_2–MeOH); $[\alpha]_D$ –88.0 (c 0.9,

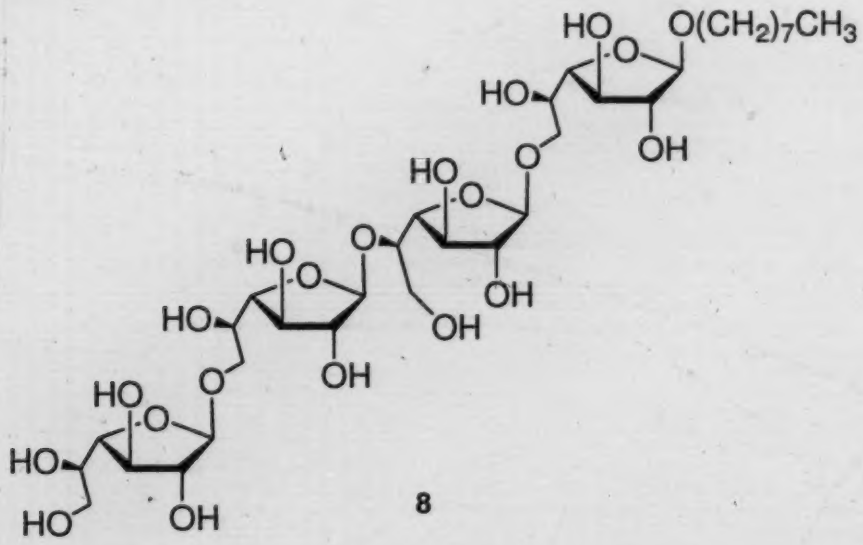
MeOH); ^{1}H NMR (500 MHz, D_2O, δ_H) 5.21 (d, 1 H, J = 1.9 Hz, H-1''), 5.00 (s, 1 H, H-1'), 4.98 (d, 1 H, J = 2.2 Hz, H-1), 4.16 (dd, 1 H, J = 4.0, 1.9 Hz, H-2"), 4.14–4.13 (m, 2 H, H-2', H-3'), 4.10–4.04 (m, 4 H, H-3", H-4', H-3, H-2), 4.02 (dd, 1 H, J = 4.0, 3.0 Hz, H-4"), 3.96–3.94 (m, 3 H, H-4, H-5', H-5), 3.86–3.71 (m, 6 H, H-6_a, H-6_b, H-6_a', H-6_b', H-6_a", H-5"), 3.66 (dd, 1 H, J = 12.0, 8.0 Hz, H-6_b"), 3.60 (ddd, 1 H, J = 10.0, 7.0, 7.0 Hz, octyl O*C*H_2), 3.55 (ddd, 1 H, J = 10.0, 7.0, 7.0 Hz, octyl O*C*H_2), 1.60–1.37 (m, 2 H, octyl *C*H_2), 1.37–1.25 (m, 10 H, octyl *C*H_2), 0.88 (t, 3 H, J = 7.0 Hz, octyl *C*H_3); ^{13}C NMR (125 MHz, D_2O δ_C) 108.6 (C-1'), 108.0 (C-1), 107.9 (C-1''), 83.5 (C-4'), 83.3 (C-4), 82.7 (C-4"), 82.1 (C-2"), 82.0 (C-2'), 81.9 (C-2), 77.5 (C-3), 77.4 (C-3"), 77.38, (C-3), 76.8 (C-5'), 71.4 (C-5), 70.2 (C-5"), 70.1 (C-6), 69.4 (octyl O*C*H_2), 63.8 (C-6"), 61.9 (C-6'), 32.0 (octyl *C*H_2), 29.6 (octyl *C*H_2), 29.3 (octyl *C*H_2), 29.3 (octyl *C*H_2), 26.1 (octyl *C*H_2), 22.9 (octyl *C*H_2), 14.5 (octyl *C*H_3). ESI-MS *m/z* calcd. for (M + Na) $C_{26}H_{48}O_{16}Na$: 639.2837. Found: 639.2835.



7

Octyl β-D-galactofuranosyl-(1→5)-β-D-galactofuranosyl-(1→6)-β-D-galactofuranosyl-(1→5)-β-D-galactofuranoside (7). Using the general deprotection procedure, octyl glycoside **92** (11.0 mg, 0.005 mmol) gave a crude product, which was purified by column chromatography (3:1 CH_2Cl_2–MeOH). The solvent was evaporated and the residue was redissolved in water before filtration through a Sep-pak C-18 cartridge to furnish **7** as a clear, colorless oil (3.3 mg, 82%). R_f 0.21 (3:1 CH_2Cl_2–MeOH); $[\alpha]_D$ –67.4 (*c* 0.2, MeOH); ^{1}H NMR (600 MHz, D_2O, δ_H) 5.23 (d, 1 H, J = 1.8 Hz, H-1'''), 5.22 (d, 1 H, J = 2.0 Hz, H-1'), 5.01 (s, 1 H, H-1''), 4.96 (s, 1 H, J = 1.0 Hz, H-1), 4.14–3.94 (m, 12 H, H-2', H-2'', H-2''', H-3', H-3'', H-3''', H-2, H-3, H-4, H-4', H-4'', H-4''') 3.61–3.75 (m, 7 H, H-5', H-5'', H-6_a', H-6_a'', H-6_a''', H-6_b''', H-5'''), 3.74–3.61 (m, 6 H, H-5, H-6_a, octyl OCH_2, H-6_b',H-6_b'', H-6_b), 3.54 (ddd, 1 H, J = 12.0, 7.5, 7.5 Hz, octyl OCH_2), 1.60–1.37 (m, 2 H, octyl CH_2), 1.37–1.25 (m, 10 H, octyl CH_2), 0.88 (t, 3 H, J = 7.0 Hz, octyl CH_3); ^{13}C NMR (125 MHz, D_2O δ_C) 110.6 (C-1'''), 109.9, 109.8 (C-1'', C-1'), 109.7 (C-1), 85.8 (C-4''), 85.4 (C-4'''), 84.6 (C-2'''), 84.2 (C-2''),

84.04 (C-4), 84.00 (C-4'), 83.9 (C-2'), 83.8 (C-2), 79.5 (C-3'''), 79.4 (C-3'), 79.3 (C-3''), 78.7 (C-3), 78.6 (C-5''), 73.3 (C-5, C-5'''), 72.3 (C-5'), 72.1 (C-6'), 69.4, (octyl OCH_2), 65.6 (C-6), 64.0 (C-6''), 63.8 (C-6'''), 34.8 (octyl CH_2), 34.0 (octyl CH_2), 32.6 (octyl CH_2), 31.5 (octyl CH_2), 25.5 (octyl CH_2), 24.8 (octyl CH_2), 16.2 (octyl CH_3). ESI-MS *m/z* calcd. for (M + Na) $C_{32}H_{58}O_{21}Na$: 801.3362. Found: 801.3363.

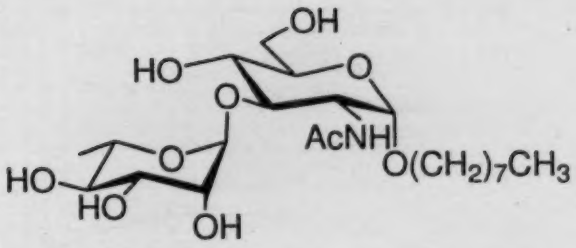


8

Octyl β-D-galactofuranosyl-(1→6)-β-D-galactofuranosyl-(1→5)-β-D-galactofuranosyl-(1→6)-β-D-galactofuranoside (8). Using the general deprotection procedure, octyl glycoside **95** (25.0 mg, 0.01 mmol) gave a crude product, which was purified by column chromatography (3:1 CH_2Cl_2–MeOH). The solvent was evaporated and the residue was redissolved in water before filtration through a Sep-pak C-18 cartridge to furnish **8** as a clear, colorless oil (5.4 mg, 70%). R_f 0.22 (3:1 CH_2Cl_2–MeOH,); $[\alpha]_D$ –143.3 (*c* 0.1, MeOH); ^{1}H NMR (600 MHz, D_2O, δ_H) 5.22 (d, 1 H, J = 1.3 Hz, H-1''), 5.04 (d, 1 H, J = 1.5 Hz, H-1'''), 5.00 (s, 1 H, H-1'), 4.95 (s, 1 H, H-1), 4.14–

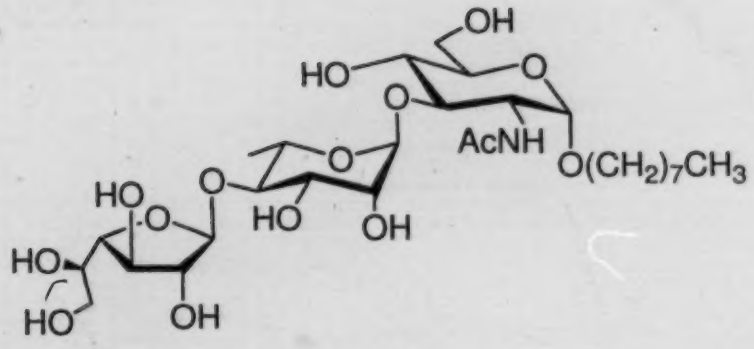
3.94 (m, 14 H, H-2", H-2''', H-2', H-3', H-3", H-3''',H-4', H-3, H-2, H-4", H-4, H-5', H-5, H-4'''), 3.90–3.71 (m, 10 H, H-6_a, H-6_b, H-6_a', H-6_b', H-6_a", H-5", H-6_a''', H-5''', H-6_b''', H-6_b"), 3.60 (ddd, 1 H, J = 10.0, 7.0, 7.0 Hz, octyl OCH_2), 3.55 (ddd, 1 H, J = 10.0, 7.0, 7.0 Hz, octyl OCH_2), 1.60–1.57 (m, 2 H, octyl CH_2), 1.37–1.25 (m, 10 H, octyl CH_2), 0.88 (t, 3 H, J = 7.0 Hz, octyl CH_3); ^{13}C NMR (125 MHz, D_2O, δ_C) 108.8 (C-1'''), 108.7 (C-1'), 108.0 (C-1), 107.9 (C-1''), 83.9 (C-4'''), 83.83 (C-2'''), 83.81 (C-4'), 83.3 (C-4), 82.8 (C-4"), 82.1 (C-2''), 81.9 (C-2), 77.6 (C-3'''), 77.6 (C-3'), 77.5 (C-3''), 77.4 (C-3), 76.7 (C-5'), 71.72 (C-5'''), 71.69 (C-5), 70.4 (C-5''), 70.0 (C-6''), 69.4, (octyl OCH_2), 63.6 (C-6), 63.6 (C-6'''), 61.9 (C-6'), 32.0 (octyl CH_2), 29.0 (octyl CH_2), 29.6 (octyl CH_2), 29.3 (octyl CH_2), 26.1 (octyl CH_2), 22.9 (octyl CH_2), 14.5 (octyl CH_3). ESI-MS *m/z* calcd. for (M + Na) $C_{32}H_{58}O_{21}Na$: 801.3362. Found: 801.3363.

3.3 General Procedure for the Deprotection of Perbenzoylated Intermediates Leading to Compounds 9-12. The starting material was dissolved in 4:1 HOAc–H_2O (10–30 mL) and heated at 70 °C for 4 h. The solvent was evaporated upon completion of the reaction. The residue was dissolved in MeOH (10–50 mL) followed by dropwise addition of NaOMe in MeOH (0.1 M) until the pH of the solution was 12. The reaction mixture was then stirred at rt for 4 h and was neutralized by the addition of Amberlyst-15 (H^+) cation exchange resin. The solution was filtered and the filtrate concentrated to give a syrupy residue. The resulting crude product was then purified by column chromatography. The solvent was evaporated and the residue was redissolved in water before filtration through a Sep-pak C-18 cartridge.



9

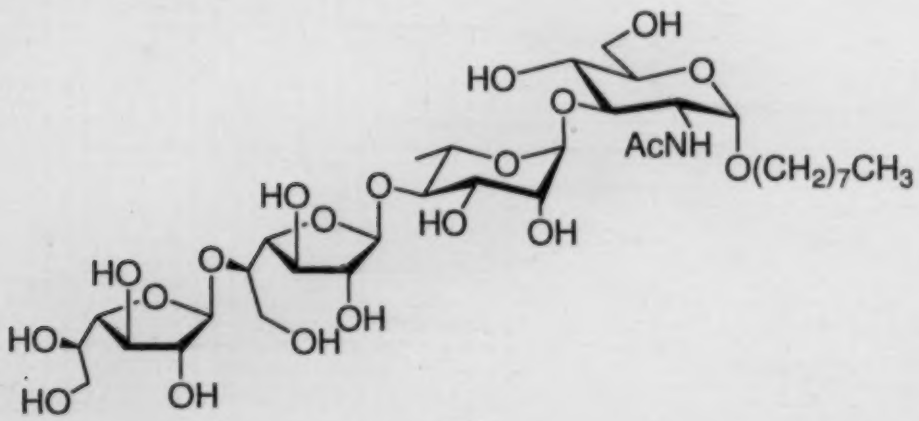
Octyl α-L-rhamnopyranosyl-(1→3)-2-acetamido-2-deoxy-α-D-glucopyranoside (9). Using the general deprotection procedure, octyl glycoside **114** (58.0 mg, 0.08 mmol) gave a crude product, which was purified by column chromatography (10:1 CH_2Cl_2–MeOH). The solvent was evaporated and the residue was redissolved in water before filtration through a Sep-pak C-18 cartridge to afford disaccharide **9** as a white solid (22.0 mg, 55%). R_f 0.54 (6:1 CH_2Cl_2–MeOH); $[\alpha]_D$ –20.1 (*c* 1.0, MeOH); ^{1}H NMR (500 MHz, D_2O, δ_H) 4.86 (d, 1 H, J = 1.6 Hz, H-1'), 4.82 (d, 1 H, J = 3.6 Hz, H-1), 4.05 (dd, 1 H, J = 10.4, 3.6 Hz, H-2), 3.98–3.68 (m, 1 H, H-5'), 3.86 (dd, 1 H, J = 10.0, 2.2 Hz, H-3'), 3.81–3.68 (m, 6 H, H-3, H-5, H-6_a, H-4, octyl OCH_2, H-2'), 3.57–3.48 (m, 2 H, H-6_b, octyl OCH_2), 3.45 (dd, 1 H, J = 10.0, 10.0 Hz, H-4'), 2.06 (s, 3 H, C(=O)CH_3), 1.64–1.60 (m, 2 H, octyl CH_2), 1.38–1.23 (m, 13 H, octyl CH_2, H-6'), 0.85 (t, 3 H, J = 7.0 Hz, octyl CH_3); ^{13}C NMR (125 MHz, D_2O, δ_C) 170.9 (C=O), 102.1 (C-1'), 97.8 (C-1), 80.4 (C-2'), 72.9 (C-4'), 72.7 (C-3'), 71.6 (C-4), 71.1 (C-3), 69.7 (C-5'), 69.2 (C-5), 69.0 (octyl OCH_2), 61.4 (C-6), 54.2 (C-2), 32.1 (octyl CH_2), 29.44 (octyl CH_2), 29.40 (2 x octyl CH_2), 26.3 (octyl CH_2), 23.0 (octyl CH_2), 22.8 (C(=O)CH_3), 17.4 (C-6'), 14.3 (octyl CH_3). ESI-MS *m/z* calcd. for (M + Na) $C_{22}H_{41}NO_{10}Na$: 502.2623. Found: 502.2621.



10

Octyl β-D-galactofuranosyl-(1→4)-α-L-rhamnopyranosyl-(1→3)-2-acetamido-2-deoxy-α-D-glucopyranoside (10). Using the general deprotection procedure, octyl glycoside **115** (57.3 mg, 0.05 mmol) gave a crude product, which was purified by column chromatography (4:1 CH_2Cl_2–MeOH). The solvent was evaporated and the residue was redissolved in water before filtration through a Sep-pak C-18 cartridge to afford trisaccharide **10** as a white solid (27.2 mg, 89%). R_f 0.36 (3:1 CH_2Cl_2–MeOH); $[\alpha]_D$ –13.2 (*c* 0.1, MeOH); ^{1}H NMR (500 MHz, D_2O, δ_H) 5.29 (br s, 1 H, H-1''), 4.87 (s, 1 H, H-1'), 4.80 (d, 1 H, J = 3.5 Hz, H-1), 4.11 (dd, 1 H, J = 4.0, 1.8 Hz, H-2''), 4.08–3.99 (m, 4 H, H-3", H-4", H-5', H-2), 3.88–3.48 (m, 13 H, H-3, H-4, H-5, H-6_a, H-6_b, H-2', H-3', H-4', H-5", H-6_a", H-6_b", octyl OCH_2), 2.06 (s, 3 H, C(=O)CH_3), 1.64–1.60 (m, 2 H, octyl CH_2), 1.38–1.23 (m, 13 H, octyl CH_2, H-6'), 0.85 (t, 3 H, J = 7.0 Hz, octyl CH_3); ^{13}C NMR (125 MHz, D_2O, δ_C) 174.9 (C=O), 109.1 (C-1"), 101.9 (C-1'), 97.7 (C-1), 83.6 (C-4"), 82.0 (C-2"), 80.5 (C-2'), 78.7 (C-4'), 77.6 (C-3"), 72.9 (C-4), 71.9 (C-3'), 71.5 (C-3), 71.4 (C-5"), 69.1 (C-5'), 69.0 (octyl OCH_2), 68.3 (C-5), 63.7 (C-6"), 61.4 (C-6), 54.2 (C-2), 32.1 (octyl CH_2), 29.42 (octyl CH_2), 29.40 (2 x octyl CH_2), 26.3 (octyl CH_2),

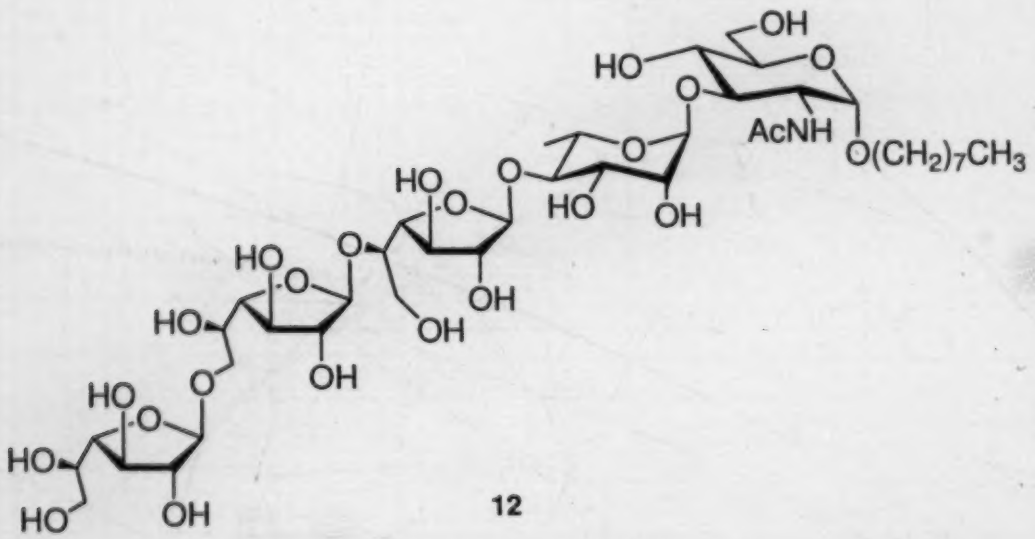
22.9 (octyl CH_2), 22.8 (C(=O)CH_3), 17.4 (C-6'), 14.3 (octyl CH_3). ESI-MS *m/z* calcd. for (M + Na) $C_{28}H_{51}NO_{15}Na$: 664.3151. Found: 664.3153.



11

Octyl β-D-galactofuranosyl-(1→5)-β-D-galactofuranosyl-(1→4)-α-L-rhamnopyranosyl-(1→3)-2-acetamido-2-deoxy-α-D-glucopyranoside (11). Using the general deprotection procedure, octyl glycoside **116** (60.0 mg, 0.04 mmol) gave a crude product, which was purified by column chromatography (4:1 CH_2Cl_2–MeOH). The solvent was evaporated and the residue was redissolved in water before filtration through a Sep-pak C-18 cartridge to afford tetrasaccharide **11** as a white solid (21.5 mg, 68%). R_f 0.21 (3:1 CH_2Cl_2–MeOH); $[\alpha]_D$ –11.7 (*c* 0.2, MeOH); ^{1}H NMR (600 MHz, D_2O, δ_H) 5.27 (d, 1 H, *J* = 1.8 Hz, H-1''), 5.22 (d, 1 H, *J* = 1.8 Hz, H-1'''), 4.86 (d, 1 H, *J* = 1.8 Hz, H-1'), 4.80 (d, 1 H, *J* = 3.6 Hz, H-1), 4.15 (dd, 1 H, *J* = 3.6, 1.8 Hz, H-2'''), 4.13–4.02 (m, 7 H, H-3''', H-4''', H-2'', H-3'', H-4'', H-5', H-2), 3.96 (dd, 1 H, *J* = 8.5, 8.5 Hz, H-3), 3.89–3.63 (m, 15 H, H-4, H-5, H-6_a, H-6_b, H-2', H-3', H-4', H-5'', H-6_a'', H-6_b'', H-5''', H-6_a''', H-6_b''', 2 × octyl OCH_2), 2.06 (s, 3 H, C(=O)CH_3), 1.64–1.60 (m, 2 H,

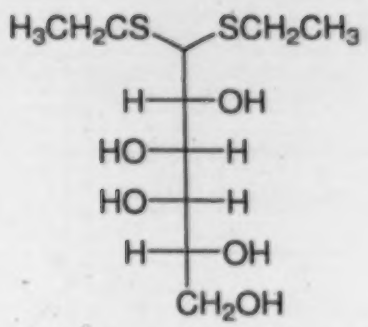
octyl CH_2). 1.38–1.23 (m, 13 H, octyl CH_2, H-6'), 0.85 (t, 3 H, J = 7.0 Hz, octyl CH_3); ^{13}C NMR (125 MHz, D_2O, δ_C) 174.9 (C=O), 109.3 (C-1''), 108.1 (C-1'''), 102.1 (C-1'), 97.7 (C-1), 83.5 (C-4''), 82.5 (C-2'''), 82.4 (C-4'''), 82.1 (C-2''), 80.5 (C-2'), 79.2 (C-4'), 77.4 (C-3''), 77.3 (C-3'''), 76.6 (C-3'), 72.9 (C-4), 71.9 (C-3), 71.5 (C-5''), 71.4 (C-5'''), 69.2 (C-5'), 69.0 (octyl OCH_2), 68.2 (C-5), 63.7 (C-6''), 62.1 (C-6'''), 61.4 (C-6), 54.2 (C-2), 32.0 (octyl CH_2), 29.42 (octyl CH_2), 29.40 (octyl CH_2), 29.4 (octyl CH_2), 26.3 (octyl CH_2), 22.9 (octyl CH_2), 22.8 ($C(=O)CH_3$), 17.4 (C-6'), 14.3 (octyl CH_3). ESI-MS m/z calcd. for (M + Na) $C_{34}H_{61}NO_{20}Na$: 826.3679. Found: 826.3682.



12

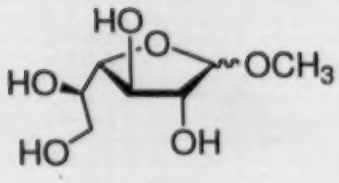
Octyl β-D-galactofuranosyl-(1→6)-β-D-galactofuranosyl-(1→5)-β-D-galactofuranosyl-(1→4)-α-L-rhamnopyranosyl-(1→3)-2-acetamido-2-deoxy-α-D-glucopyranoside (12). Using the general deprotection procedure, octyl glycoside **117** (46.0 mg, 0.02 mmol) gave a crude product which was purified by column chromatography (4:3:3:2 EtOAc–AcOH–CH_3OH–H_2O). The solvent was evaporated and

the residue was redissolved in water and lyophilized. The residue obtained was filtered through a Sep-pak C-18 cartridge to afford pentasaccharide **12** as a white solid (12.0 mg, 62%). $[\alpha]_D$ –2.9 (*c* 0.1, MeOH); ^{1}H NMR (600 MHz, D_2O, δ_H) 5.26 (d, 1 H, *J* = 1.8 Hz, H-1''), 5.20 (d, 1 H, *J* = 1.8 Hz, H-1'''), 5.04 (d, 1 H, *J*= 1.8 Hz, H-1''''), 4.86 (s, 1 H, H-1'), 4.79 (br s, 1 H, H-1), 4.14–3.94 (m, 13 H, H-2''', H-2'', H-3'''', H-2'''', H-2, H-3'', H-3''', H-4''', H-4'''', H-5', H-5'', H-3, H-4), 3.89–3.45 (m, 17 H, H-2', H-3', H-4', H-4'', H-6$_a$, H-6$_b$, H-5, H-6$_a$'', H-6$_b$'', H-5''', H-6$_a$''', H-6$_b$''', H-5'''', H-6$_a$'''', H-6$_b$'''', 2 x octyl OCH_2), 2.06 (s, 3 H, C(=O)CH_3), 1.64–1.60 (m, 2 H, octyl CH_2), 1.38–1.23 (m, 13 H, octyl CH_2, H-6'), 0.85 (t, 3 H, *J* = 7.0 Hz, octyl CH_3); ^{13}C NMR (125 MHz, D_2O, δ_C) 174.9 (C=O), 109.3 (C-1''), 108.7 (C-1''''), 108.1 (C-1'''), 102.1 (C-1'), 97.7 (C-1), 83.84 (C-4''), 83.80 (C-4''''), 82.6 (C-2'''), 82.5 (C-4'''), 82.1 (C-2''), 81.8 (C-2''''), 80.5 (C-2'), 79.2 (C-4'), 77.6 (C-3''), 77.5 (C-3'''), 77.4 (C-3''''), 76.5 (C-3'), 72.9 (C-4), 71.9 (C-3), 71.7 (C-5'''') 71.5 (C-5''), 70.4 (C-5'''), 70.2 (C-6''''), 69.2 (C-5'), 69.0 (octyl OCH_2), 68.3 (C-5), 63.6 (C-6''), 62.1 (C-6'''), 61.4 (C-6), 54.2 (C-2), 32.0 (octyl CH_2), 29.4 (octyl CH_2), 29.4 (octyl CH_2), 29.4 (octyl CH_2), 26.3 (octyl CH_2), 22.9 (octyl CH_2), 22.8 (C(=O)CH_3), 17.4 (C-6'), 14.3 (octyl CH_3). ESI-MS *m/z* calcd. for (M + Na)$C_{40}H_{71}NO_{25}Na$: 988.4207. Found: 988.4211.



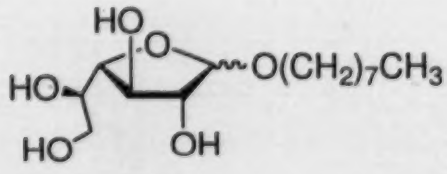
34

D-galactose diethyl dithioacetal (34). D-galactose (50.00 g, 0.278 mol) was dissolved at rt in concentrated HCl (75 mL). Ethanethiol (50 mL) was then added, and the reaction mixture was shaken vigorously, releasing the pressure occasionally. After 3–5 minutes, a definite temperature increase was noted; a little amount of ice and ice water was then added. More ice water was added and the crystalline product formed was filtered and washed with small amount of ice water. Recrystallization from absolute ethanol afforded a pure white crystalline **34** (37.00 g, 47% yield). ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 4.13 (dd, 1 H, J = 9.0, 1.5 Hz, H-5), 4.07 (d, 1 H, J = 10.0 Hz, H-1), 3.91 (dd, 1 H, J = 9.0, 1.5 Hz, H-3), 3.88 (dd, 1 H, 1 H, J = 10.0, 1.5 Hz, H-2), 3.64 (d, 1 H, J = 9.0 Hz, H-4), 3.62 (d, 2 H, J = 7.0 Hz, H-6_a, H-6_b), 2.77–2.65 (m, 4 H, 2 x SCH_2), 1.27–1.23 (m, 6 H, 2 x CH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 72.8, 71.9, 71.8, 71.1 (C-2, C-3, C-4, C-5), 65.1 (C-6), 56.4 (C-1), 25.4, 25.4 (2 x ethyl CH_2), 14.9, 14.8 (2 x ethyl CH_3). ESI-MS *m/z* calcd. for (M + Na) $C_{10}H_{22}O_5S_2Na$: 309.0806. Found: 309.0897.



35

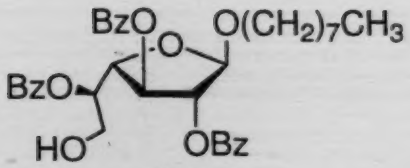
Methyl α/β-D-galactofuranoside (35). Dithioacetal **34** (5.90 g, 0.14 mol) was dissolved in 2% I_2 in MeOH (w/v, 295 mL) at rt and stirred for 7 h. Excess I_2 was quenched by the increment addition of solid $Na_2S_2O_3$ until the I_2 color diminished. This was followed by the addition of $NaHCO_3$ to neutralize the reaction mixture. Evaporation of the solvent gave **35** (β/α = 9:1) as a yellow solid (21.20 g, 78%). R_f 0.34 (6:1 CH_2Cl_2–MeOH); β-isomer (isolated pure after column chromatography): $[\alpha]_D$ –20.6 (*c* 1.1, $CHCl_3$); ^{1}H NMR (400 MHz, D_2O, δ_H) 4.83 (s, 1 H, H-1), 3.98–3.92 (m, 2 H, H-4, H-5), 3.88 (dd, 1 H, *J* = 4.3, 4.3 Hz, H-3), 3.77–3.73 (m, 1 H, H-2), 3.66–3.64 (m, 2 H, H-6_a, H-6_b), (3.45 (s, 3 H, OC*H*$_3$); ^{13}C NMR (100 MHz, D_2O, δ_C) 108.6 (C-1), 83.3 (C-2), 81.6 (C-4), 71.3 (C-3), 71.1 (C-5), 63.2 (C-6), 55.4 (O*C*H$_3$). ESI-MS *m/z* calcd. for (M + Na) $C_7H_{14}O_6Na$: 217.0688. Found: 217.0758.



38

Octyl β/α-D-galactofuranoside (38). Dithioacetal **34** (5.90 g, 140 mmol) was dissolved in 5% octanol (w/v, 200 mL) at rt and stirred for 24 h. Excess I_2 was quenched by adding

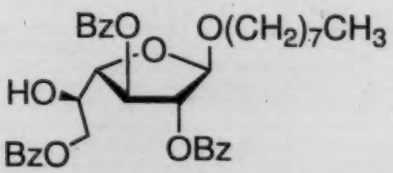
a saturated aqueous solution of $Na_2S_2O_3$ (10 mL). This was followed by the addition of a saturated aqueous solution of $NaHCO_3$ to neutralize the reaction mixture. The aqueous layer was washed twice with EtOAc and the combined organic layers were concentrated to give a syrup that was purified by column chromatography (9:1 CH_2Cl_2–MeOH) to give **38** (β/α = 9:1) as a white solid. R_f 0.23 (α), 0.17 (β) (9:1 CH_2Cl_2–MeOH); β: ^{1}H NMR (500 MHz, CD_3OD, δ_H) 4.83 (d, 1 H, J = 1.5 Hz, H-1), 3.98 (dd, 1 H, J = 4.0, 4.0 Hz, H-3), 3.92 (dd, 1 H, J = 4.0, 1.5 Hz, H-2), 3.89 (dd, 1 H, J = 6.0, 4.0 Hz, H-4), 3.72–3.66 (m, 2 H, H-5, octyl OCH_2), 3.62–3.60 (m, 2 H, H-6_a, H-6_b), 3.40 (ddd, 1 H, J = 9.6, 6.5, 6.5 Hz, octyl OCH_2), 1.60–1.52 (m, 2 H, octyl CH_2), 1.38–1.22 (m, 10 H, octyl CH_2), 0.90 (t, 3 H, J = 7.0 Hz, octyl CH_3); ^{13}C NMR (125 MHz, CD_3OD, δ_C) 109.4 (C-1), 84.2 (C-2), 83.5 (C-4), 78.8 (C-3), 72.5 (C-5), 65.0 (octyl OCH_2), 64.6 (C-6), 33.0 (octyl CH_2), 30.7 (octyl CH_2), 30.5 (octyl CH_2), 30.4 (octyl CH_2), 27.3 (octyl CH_2), 23.7 (octyl CH_2), 14.4 (octyl CH_3); α: ^{1}H NMR (500 MHz, CD_3OD, δ_H) 4.84 (d, 1 H, J = 4.5 Hz, H-1), 4.09 (dd, 1 H, J = 5.0, 4.0 Hz, H-3), 3.94 (dd, 1 H, J = 4.0, 4.5 Hz H-2), 3.80 (ddd, 1 H, J = 9.5, 6.9, 6.9 Hz, octyl OCH_2), 3.72 (dd, 1 H, J = 5.0, 5.0 Hz, H-4), 3.65–3.53 (m, 3 H, H-5, H-6_a, H-6_b), 3.46 (ddd, 1 H, J = 9.5, 6.9, 6.9 Hz, octyl OCH_2); 1.63–1.60 (m, 2 H, octyl CH_2), 1.32–1.28 (m, 10 H, octyl CH_2), 0.89 (t, 3 H, J = 6.8 Hz, octyl CH_3); ^{13}C NMR (125 MHz, CD_3OD, δ_C) 103.5 (C-1), 84.3 (C-2), 79.5 (C-4), 77.1 (C-3), 75.2 (C-5), 70.5 (octyl OCH_2), 64.9 (C-6), 33.2 (octyl CH_2), 31.5 (octyl CH_2), 31.3 (octyl CH_2), 31.2 (octyl CH_2), 28.3 (octyl CH_2), 24.3 (octyl CH_2), 14.2 (octyl CH_3). ESI-MS *m/z* calcd. for (M+Na) $C_{14}H_{28}O_6Na$: 315.1784. Found: 315.1834.



39

Octyl 2,3,5-tri-*O*-benzoyl-β-D-galactofuranoside (39). Method 1: To a solution of **76** (0.28 g, 0.33 mmol) in CH_2Cl_2–MeOH (3:1, 7 mL), *p*-toluenesulfonic acid monohydrate (0.06 g, 0.33 mmol) was added. The reaction mixture was stirred for 3 h and diluted with CH_2Cl_2 (5 mL). The organic layer was washed with a saturated aqueous solution of $NaHCO_3$, water, dried and concentrated to give a colorless syrup. The residue was purified by column chromatography (3:1 hexanes–EtOAc) to give **39** (0.18 g, 89%) as a fluffy semi-solid. R_f 0.48 (3:1 hexanes–EtOAc); $[\alpha]_D$ –12.0 (*c* 1.0, $CHCl_3$); Method 2: Compound **76** (1.36 g, 1.61 mmol) was dissolved in CH_2Cl_2 (75 mL), cooled to 0 °C, and trifluoroacetic acid (5% in CH_2Cl_2, 4.95 mL) was added dropwise. After stirring for 8 h, the reaction mixture was co-evaporated with toluene and concentrated to give a syrupy residue that was purified by column chromatography (8:1 hexanes–EtOAc) to give **39** (409 mg, 42%) as a colorless syrup. R_f 0.48 (3:1 hexanes–EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$, δ_H) 8.08–7.80 (m, 6 H, Ar), 7.64–6.90 (m, 9 H, Ar), 5.64 (ddd, 1 H, J = 6.0, 6.0, 4.5 Hz, H-5), 5.60 (d, 1 H, J = 5.0 Hz, H-3), 5.47 (d, 1 H, J = 1.1 Hz, H-2), 5.32 (br s, 1 H, H-1), 4.64 (dd, 1 H, J = 5.0, 4.5 Hz, H-4), 4.10–4.05 (d, 2 H, H-6_a, H-6_b), 3.77 (ddd, 1 H, J = 9.5, 7.0, 7.0 Hz, octyl OCH_2), 3.58 (ddd, 1 H, J = 9.5, 7.0, 7.0 Hz, octyl OCH_2), 1.64–1.61 (m, 2 H, octyl CH_2), 1.46–1.21 (m, 10 H, octyl CH_2), 0.88 (t, 3 H, J = 7.0 Hz, octyl CH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ_H) 166.4 (C=O), 165.9 (C=O), 165.5 (C=O), 133.5 (Ar), 133.4 (Ar), 130.2 (Ar), 129.95 (Ar), 129.86 (Ar), 129.6 (Ar), 129.1 (Ar), 128.5 (Ar), 128.44 (Ar), 128.35 (Ar), 105.7 (C-1), 82.2 (C-2), 82.0 (C-3), 77.7 (C-4),

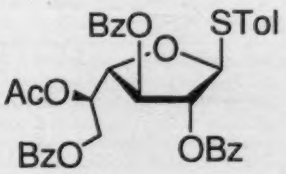
73.6 (C-5), 67.7 (octyl OCH_2), 62.7 (C-6), 31.8 (octyl CH_2), 29.5 (octyl CH_2), 29.4 (octyl CH_2), 29.3 (octyl CH_2), 26.2 (octyl CH_2), 22.7 (octyl CH_2), 14.1 (octyl CH_3). ESI-MS *m/z* calcd. for (M+Na) $C_{35}H_{40}O_9$: 627.2592. Found: 627.2588.



40

Octyl 2,3,6-tri-*O*-benzoyl-β-D-galactofuranoside (40). Method 1: Prepared from **75** (1.00 g, 2.00 mmol) in pyridine (15 mL) and benzoyl chloride (0.06 mL, 5.0 mmol) as described for **57**. The crude product was purified by column chromatography (4:1 hexanes–EtOAc) to give **40** (1.08 g, 90%) as a colorless syrup. R_f 0.53 (3:1 hexanes–EtOAc); Method 2: Compound **76** (1.36 g, 1.61 mmol) was dissolved in CH_2Cl_2 (75 mL), cooled to 0 °C, and trifluoroacetic acid (5% in CH_2Cl_2, 4.95 mL) was added dropwise. After stirring for 8 h, the reaction mixture was co-evaporated with toluene and concentrated to give a syrupy residue that was purified by column chromatography (8:1 hexanes–EtOAc) to give **40** (338 mg, 35%) as colorless syrup. R_f 0.53 (3:1 hexanes–EtOAc); $[\alpha]_D$ –20.3 (*c* 1.1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 8.08–7.60 (m, 6 H, Ar), 7.55–7.10 (m, 9 H, Ar), 5.64 (d, 1 H, J = 4.8 Hz, H-3), 5.52 (d, 1 H, J = 1.3 Hz, H-2), 5.27 (s, 1 H, H-1), 4.61 (dd, 1 H, J = 12.0, 7.9 Hz, H-6_a), 4.55–4.40 (m, 2 H, H-5, H-6_b), 4.38 (dd, 1 H, J = 4.8, 2.0 Hz, H-4), 3.72 (ddd, 1 H, J = 9.5, 6.7, 6.7 Hz, octyl OCH_2), 3.50 (ddd, 1 H, J = 9.5, 6.7, 6.7 Hz, octyl OCH_2), 1.63–1.59 (m, 2 H, octyl CH_2), 1.35–0.95 (m, 10 H, octyl CH_2), 0.88 (t, 3 H, J = 6.6 Hz, octyl

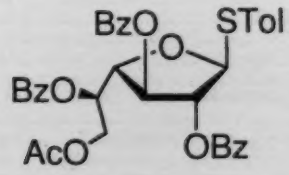
CH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 166.5 (C=O), 166.1 (C=O), 165.4 (C=O), 133.5 (Ar), 133.1 (Ar), 130.0 (Ar), 129.94 (Ar), 129.87 (Ar), 129.85 (Ar), 129.7 (Ar), 129.2 (Ar), 129.1 (Ar), 128.53 (Ar), 128.46 (Ar), 128.3 (Ar), 105.7 (C-1), 83.1 (C-2), 81.5 (C-4), 78.2 (C-3), 69.1 (C-5), 67.7 (octyl OCH_2), 61.7 (C-6), 31.8 (octyl CH_2), 29.5 (octyl CH_2), 29.4 (octyl CH_2), 29.3 (octyl CH_2), 26.2 (octyl CH_2), 22.6 (octyl CH_2), 14.1 (octyl CH_3). ESI-MS *m/z* calcd. for (M+Na) $C_{35}H_{40}O_9Na$: 627.2592. Found: 627.2589.



41

***p*-Tolyl 5-*O*-acetyl-2,3,6-tri-*O*-benzoyl-1-thio-β-D-galactofuranoside (41).** To a solution of **62** (0.23 g, 0.38 mmol) in pyridine (10 mL), was added acetic anhydride (50 μL, 0.37 mmol). The reaction mixture was stirred for 2 h, poured into an ice–water mixture, and extracted with CH_2Cl_2. The combined organic layers were washed with brine, dried and concentrated. Co-evaporation with toluene removed all traces of pyridine and the residue was purified by column chromatography (4:1 hexanes–EtOAc) to give **41** as a white solid (0.22 g, 90%). R_f 0.36 (3:1 hexanes–EtOAc); $[\alpha]_D$ –61.2 (*c* 0.5, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$, δ_H) 8.10–8.06 (m, 4 H, Ar), 8.00–7.98 (m, 2 H, Ar), 7.62–7.44 (m, 7 H, Ar), 7.36–7.32 (m, 4 H, Ar), 7.14 (d, 2 H, J = 9.0 Hz, Ar), 5.78 (ddd, 1 H, J = 6.9, 6.9, 4.4 Hz, H-5), 5.77 (br s, 1 H, H-1), 5.64 (dd, 1 H, J = 1.6, 1.1 Hz, H-2), 5.63 (dd, 1 H, J = 4.7, 1.6 Hz, H-3), 4.86 (dd, 1 H, J = 4.7, 4.4 Hz, H-4), 4.68 (dd, 1 H, J = 11.8, 5.7 Hz, H-6_a), 4.56 (dd, 1 H, J = 11.8, 5.7 Hz, H-6_b), 2.34 (s, 3 H, ArCH_3),

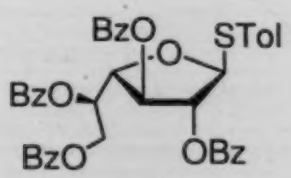
2.01 (s, 3 H, C(=O)CH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 170.0 (C=O), 166.0 (C=O), 165.4 (C=O), 165.3 (C=O), 138.3 (Ar), 133.6 (Ar), 133.1 (Ar), 133.0 (Ar), 130.0 (Ar), 129.9 (Ar), 129.8 (Ar), 129.7 (Ar), 129.6 (Ar), 129.3 (Ar), 129.0 (Ar), 128.9 (Ar), 128.5 (Ar), 128.4 (Ar), 91.4 (C-1), 82.0 (C-2), 81.0 (C-4), 77.4 (C-3), 69.7 (C-5), 63.2 (C-6), 21.1 (ArCH_3), 20.8 (C(=O)CH_3). ESI-MS *m/z* calcd. for (M + Na) $C_{36}H_{32}O_9SNa$: 663.1654 Found: 663.1659.



42

***p*-Tolyl 6-*O*-acetyl-2,3,5-tri-*O*-benzoyl-1-thio-β-D-galactofuranoside (42).** Prepared from **61** (0.17 g, 0.29 mmol) in pyridine (10 mL), and acetic anhydride (35.0 μL, 0.37 mmol) as described for **41**, and the residue was purified by column chromatography (4:1 hexanes–EtOAc) to afford compound **42** as a white semi-solid (0.17 g, 90%). R_f 0.38 (3:1 hexanes–EtOAc); $[\alpha]_D$ –30.3 (*c* 0.4, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 8.10–8.05 (m, 4 H, Ar), 7.90 (d, 2 H, *J* = 7.0 Hz, Ar), 7.62–7.45 (m, 7 H, Ar), 7.36–7.31 (m, 4 H, Ar), 7.14 (d, 2 H, *J* = 9.0 Hz, Ar), 5.92 (ddd, 1 H, *J* = 6.0, 6.0, 5.0 Hz, H-5), 5.75 (d, 1 H, *J* = 0.9 Hz, H-1), 5.64–5.63 (m, 2 H, H-2, H-3), 4.86 (dd, 1 H, *J* = 5.0, 3.8 Hz, H-4), 4.53 (dd, 1 H, *J* = 11.8, 6.0 Hz, H-6_a), 4.45 (dd, 1 H, *J* = 11.8, 6.0 Hz, H-6_b), 2.34 (s, 3 H, ArCH_3), 2.01 (s, 3 H, C(=O)CH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 170.5 (C=O), 165.7 (C=O), 165.5 (C=O), 165.3 (C=O), 133.6 (Ar), 133.4 (Ar), 133.3 (Ar), 133.1 (Ar), 130.0 (Ar), 129.0 (Ar), 128.8 (Ar), 128.5 (Ar), 128.4 (Ar), 128.3 (Ar), 91.5 (C-1), 82.3 (C-2),

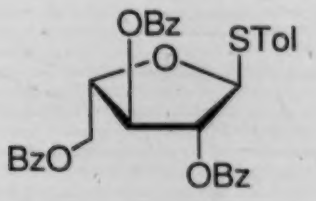
81.3 (C-4), 77.8 (C-3), 70.2 (C-5), 62.7 (C-6), 21.2 ($ArCH_3$), 20.7 ($C(=O)CH_3$);). ESI-MS *m/z* calcd. for (M + Na) $C_{36}H_{32}O_9SNa$: 663.1660. Found: 663.1659.



43

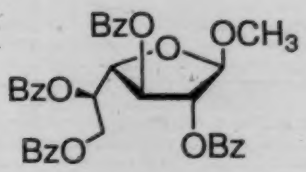
***p*-Tolyl 2,3,5,6-tetra-*O*-benzoyl-1-thio-β-D-galactofuranoside (43).** Methyl glycoside **56** (13.26 g, 21.70 mmol) was dissolved in CH_2Cl_2 (150 mL) at 0 °C and *p*-thiocresol (3.52 g, 28.3 mmol) was added. The reaction mixture was stirred for 15 min and $BF_3{\cdot}OEt_2$ (17.9 mL, 141.03 mmol) was then slowly added. The solution was then allowed to stir for 8 h while warming to rt before being neutralized with Et_3N and diluted with CH_2Cl_2. The organic layer was then washed successively a saturated aqueous solution of $NaHCO_3$, water and brine. The organic solution was dried and the solvent evaporated; the crude product was purified by chromatography (4:1 hexanes–EtOAc) to give **43** (13.57 g, 89%) as a white fluffy solid. R_f 0.38 (4:1 hexanes–EtOAc); $[\alpha]_D$ –82.0 (*c* 1.0, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$, δ_H) 8.07 (ddd, 4 H, *J* = 9.8, 8.5, 1.2 Hz, Ar), 7.96 (dd, 2 H, *J* = 8.0, 1.5 Hz, Ar), 7.89 (dd, 2 H, *J* = 8.0, 1.5 Hz, Ar), 7.60–7.45 (m, 8 H, Ar), 7.39–7.26 (m, 6 H, Ar), 7.07 (d, 2 H, *J* = 8.0 Hz, Ar), 6.10 (ddd, 1 H, *J* = 6.6, 6.6, 5.0 Hz, H-5), 5.77 (d, 1 H, *J* = 1.8 Hz, H-1), 5.71 (dd, 1 H, *J* = 5.0, 1.8 Hz, H-3), 5.66 (dd, 1 H, *J* = 1.8, 1.8 Hz, H-2), 4.95 (dd, 1 H, *J* = 5.0, 5.0 Hz, H-4), 4.75 (dd, 1 H, *J* = 11.8, 7.0 Hz, $H\text{-}6_a$), 4.71 (dd, 1 H, *J* = 11.8, 7.0 Hz, $H\text{-}6_b$), 2.32 (s, 3 H, $ArCH_3$); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 166.0 (C=O), 165.7 (C=O), 165.5 (C=O), 165.3 (C=O), 133.5 (Ar), 133.4 (Ar), 133.3 (Ar), 133.2 (Ar), 130.1 (Ar), 130.03 (Ar), 130.00 (Ar),

129.94 (Ar), 129.85 (Ar), 129.7 (Ar), 129.6 (Ar), 129.5 (Ar), 129.2 (Ar), 128.9 (Ar), 128.8 (Ar), 128.5 (Ar), 128.41 (Ar), 128.39 (Ar), 128.35 (Ar), 91.6 (C-1), 82.3 (C-2), 81.4 (C-4), 77.9 (C-3), 70.3 (C-5), 63.4 (C-6), 21.1 ($ArCH_3$). ESI-MS *m/z* calcd. for (M + Na) $C_{41}H_{34}O_9SNa$: 725.1872. Found: 725.1870.



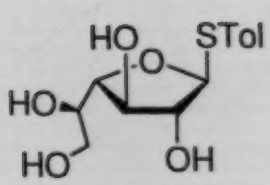
44

***p*-Tolyl 2,3, 5-tri-*O*-benzoyl-1-thio-β-L-arabinofuranoside (44).** Prepared from **67** (15.88 g, 35.60 mmol) in CH_2Cl_2 (75.0 mL) and *p*-thiocresol (4.40 g, 35.60 mmol) as described for **43** to afford **44** (18.0 g, 89%) as a white fluffy semi-solid. R_f 0.38 (4:1 hexanes–EtOAc); $[\alpha]_D$ –90.4 (c 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H): 8.14 (d, 2 H, J = 10.7 Hz, Ar), 8.06–8.00 (m, 4 H, Ar), 8.00–7.48 (m, 7 H, Ar), 7.42–7.30 (m, 4 H, Ar), 7.13 (d, 2 H, J = 8.0 Hz, Ar), 5.77 (d, 1 H, J = 1.0 Hz, H-1), 5.72 (dd, 1 H, J = 1.0, 3.0 Hz, H-2), 5.66 (dd, 1 H, J = 5.0, 3.0 Hz, H-3), 4.95 (ddd, 1 H, J = 5.0, 5.0, 5.0 Hz, H-4), 4.75 (dd, 1 H, J = 11.8, 5.0 Hz, H-5_a), 4.71 (dd, 1 H, J = 11.8, 5.0 Hz, H-5_b), 2.32 (s, 3 H, $ArCH_3$); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 166.2 (C=O), 165.6 (C=O), 165.4 (C=O), 138.2 (Ar), 133.64 (Ar), 133.56 (Ar), 133.1 (Ar), 133.0(Ar), ,130.0 (Ar), 129.91 (Ar), 129.87 (Ar), 129.77 (Ar), 129.72 (Ar), 129.6 (Ar), 129.0 (Ar), 128.9 (Ar), 128.6 (Ar), 128.5 (Ar), 128.3 (Ar), 91.7 (C-1), 82.5 (C-2), 81.0 (C-4), 78.1 (C-3), 63.6 (C-5), 21.1 ($ArCH_3$). ESI-MS *m/z* calcd. for (M + Na) $C_{33}H_{28}O_7SNa$: 591.1453. Found: 591.1510.



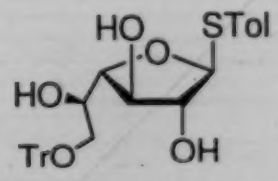
56

Methyl 2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranoside (56). To a solution of **35** (1.0 g, 5.15 mmol) in pyridine (15 mL) at 0 °C, benzoyl chloride (2.87 mL, 42.72 mmol) was added dropwise. The reaction mixture was then allowed to stir overnight while warming to rt. Ice-water was added to destroy the excess benzoyl chloride and the reaction mixture was diluted with CH_2Cl_2 (30 mL). The organic layer was then washed successively with water, two portions of 1M HCl and a saturated aqueous solution of $NaHCO_3$. The organic solution was dried and the solvent evaporated. The crude product was purified by chromatography (3:1 hexanes–EtOAc) to yield **56** (2.86 g, 91%) as a white semi-solid. R_f 0.34 (3:1 hexanes–EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 8.08–8.06 (m, 2 H, Ar), 8.05–8.01 (m, 2 H, Ar), 7.98–7.94 (m, 2 H, Ar), 7.91–7.87 (m, 2 H, Ar), 7.58–7.52 (m, 4 H, Ar), 7.41–7.37 (m, 4 H, Ar), 7.30–7.10 (m, 4 H, Ar), 6.07 (ddd, 1 H, J = 6.0, 6.0, 4.0 Hz, H-5), 5.64 (dd, 1 H, J = 5.5, 1.0 Hz, H-3), 5.45 (d, 1 H, J = 1.0 Hz, H-2), 5.21 (s, 1 H, H-1), 4.81 (dd, 1 H, J = 11.8, 6.0 Hz, H-6_a), 4.79 (dd, 1 H, J = 11.8, 6.0 Hz, H-6_b), 4.65 (dd, 1 H, J = 5.5, 4.0 Hz, H-4), 3.52 (s, 3 H, OCH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 165.1 (C=O), 165.7 (C=O), 165.7 (C=O), 165.5 (C=O), 133.5 (Ar), 133.3 (Ar), 133.2 (Ar), 133.1 (Ar), 130.1 (Ar), 129.97 (Ar), 129.93 (Ar), 129.8 (Ar), 129.7 (Ar), 129.6 (Ar), 129.5 (Ar), 129.0 (Ar), 128.5 (Ar), 128.39 (Ar), 128.36 (Ar), 106.9 (C-1), 82.2 (C-2), 81.2 (C-4), 76.5 (C-3), 70.3 (C-5), 63.5 (C-6), 55.0 (OCH_3). ESI-MS m/z calcd. for (M + Na) $C_{35}H_{30}O_{10}Na$: 633.1737. Found: 633.1286.



57

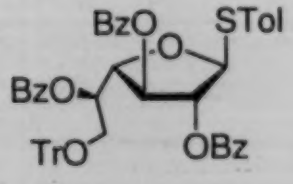
***p*-Tolyl 1-thio-β-D-galactofuranoside (57).** To a solution of **43** (15.00 g, 21.34 mmol) in 3:1 MeOH–CH_2Cl_2 was added NaOMe in MeOH (0.1 M) dropwise until the pH of the reaction mixture was 12. The solution was stirred at rt for 8 h and neutralized with glacial acetic acid. Concentration gave a yellow syrup that was purified by column chromatography (9:1 CH_2Cl_2–MeOH) to afford **57** (5.38 g, 88%) as white semi-solid. R_f 0.31 (9:1 CH_2Cl_2–MeOH); $[\alpha]_D$ –193.9 (*c* 0.3, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.40 (d, 2 H, J = 6.3 Hz, Ar), 7.10 (d, 2 H, J = 8.5 Hz, Ar), 5.19 (d, 1 H, J = 5.0 Hz, H-1), 4.09 (dd, 1 H, J = 5.4, 5.0 Hz, H-3), 3.98–3.94 (m, 2 H, H-2, H-4), 3.74 (ddd, 1 H, J = 7.0, 7.0, 5.4 Hz, H-5), 3.65–3.60 (m, 2 H, H-6_a, H-6_b), 2.28 (s, 3 H, ArCH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 138.8 (Ar), 133.7 (Ar), 132.2 (Ar), 130.7 (Ar), 93.3 (C-1), 83.1 (C-2), 83.0 (C-4), 77.1 (C-3), 72.1 (C-5), 64.6 (C-6), 21.3 (ArCH_3). ESI-MS *m/z* calcd. for (M + Na) $C_{13}H_{18}O_5SNa$: 309.0769. Found: 309.0767.



59

***p*-Tolyl-6-*O*-trityl-1-thio-β-D-galactofuranoside (59).** To a solution of **57** (0.58 g, 2.04 mmol) in pyridine (10 mL), was added trityl chloride (0.85 g, 3.05 mmol). The reaction

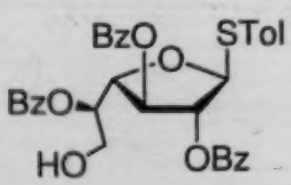
mixture was stirred at 50 °C overnight and was then poured into an ice–water mixture, and extracted with CH_2Cl_2. The combined organic layers were washed with brine and the organic solution was then dried and the solvent evaporated. Co-evaporation with toluene removed all traces of pyridine and the residue was purified by column chromatography (1:1 hexanes–EtOAc) to give **59** (0.85 g, 80%) as a colorless oil. R_f 0.12 (1:1 hexanes–EtOAc); $[\alpha]_D$ –91.9 (*c* 1.1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.46–7.44 (m, 6 H, Ar), 7.44–7.21 (m, 11 H, Ar), 7.01–6.99 (d, 2 H, J = 8.5 Hz, Ar), 5.32 (d, 1 H, J = 3.4 Hz, H-1), 4.22–4.17 (m, 2 H, H-3, H-4), 4.12 (dd, 1 H, J = 3.4, 3.4 Hz, H-2), 3.93 (dd, 1 H, J = 6.4, 6.4 Hz, H-5), 3.36 (dd, 1 H, J = 9.5, 6.4 Hz, H-6_a), 3.25 (dd, 1 H, J = 9.5, 6.4 Hz, H-6_b), 2.41 (s, 3 H, $ArCH_3$); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 143.5 (Ar), 137.7 (Ar), 132.6 (Ar), 129.9 (Ar), 129.8 (Ar), 128.7 (Ar), 127.9 (Ar), 127.2 (Ar), 92.5 (C-1), 87.1 (Ph_3C), 83.9 (C-4), 81.5 (C-2), 77.9 (C-3), 69.9 (C-5), 64.8 (C-6), 21.1 ($ArCH_3$). ESI-MS *m/z* calcd. for (M + Na) $C_{32}H_{32}O_5SNa$: 551.1865. Found: 551.1863.



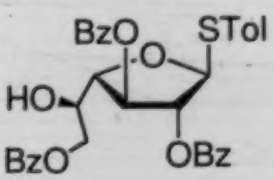
60

***p*-Tolyl 2,3,5-tri-*O*-benzoyl-6-*O*-trityl-1-thio-β-D-galactofuranoside (60).** Prepared from **59** (0.18 g, 0.33 mmol) in pyridine (5 mL) and benzoyl chloride (0.06 g, 0.21 mmol) as described for **57**. The residue was purified by column chromatography (4:1 hexanes–EtOAc) to afford **60** (0.24 g, 87%) as a white semi-solid. R_f 0.62 (3:1 hexanes–EtOAc); $[\alpha]_D$ –58.3 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 8.10 (dd, 4 H, J = 9.2, 7.2 Hz,

Ar), 7.85 (dd, 2 H, J = 7.9, 0.8 Hz, Ar), 7.63–7.18 (m, 26 H, Ar), 7.02 (d, 2 H, J = 8.4 Hz, Ar), 5.90 (ddd, 1 H, J = 6.0, 6.0, 4.5 Hz, H-5), 5.70 (d, 1 H, J = 0.7 Hz, H-1), 5.61–5.80 (m, 2 H, H-2, H-3), 5.10 (dd, 1 H, J = 4.5, 4.5 Hz, H-4), 3.57 (dd, 1 H, J = 10.1, 6.0 Hz, H-6_a), 3.43 (dd, 1 H, J = 10.0, 6.0 Hz, H-6_b), 2.32 (s, 3 H, ArCH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 165.7 (C=O), 165.4 (C=O), 165.3 (C=O), 143.6 (Ar), 138.0 (Ar), 133.5 (Ar), 133.3 (Ar), 133.2 (Ar), 133.1 (Ar), 130.0 (Ar), 129.8 (Ar), 129.7 (Ar), 129.4 (Ar), 129.1 (Ar), 128.9 (Ar), 128.6 (Ar), 128.6 (Ar), 128.5 (Ar), 128.4 (Ar), 128.4 (Ar), 127.8 (Ar), 127.8 (Ar), 127.0 (Ar), 91.3 (C-1), 86.9 (Ph_3C), 82.4 (C-4), 81.3 (C-2), 78.0 (C-3), 72.1 (C-5), 62.9 (C-6), 21.2 (ArCH_3). ESI-MS m/z calcd. for (M + Na) $C_{53}H_{44}O_8SNa$: 863.2649. Found: 863.2648.



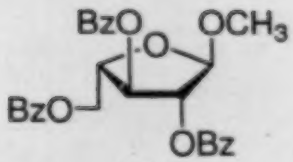
61



62

***p*-Tolyl 2,3,5-tri-*O*-benzoyl-1-thio-β-D-galactofuranoside (61)** and ***p*-Tolyl 2,3,6-tri-*O*-benzoyl-1-thio-β-D-galactofuranoside (62).** Compound **60** (0.82 g, 0.98 mmol) was dissolved in CH_2Cl_2 (30 mL), cooled to 0 °C, and trifluoroacetic acid (5% in CH_2Cl_2) (13.0 mL) was added dropwise. The reaction mixture was stirred for 12 h, co-evaporated with toluene and concentrated to give a syrup. The residue was purified by column chromatography (5:1 hexanes–EtOAc) to give **61** (0.17 g, 33%) and **62** (0.23 g, 44%) as colorless syrups. R_f 0.40 (**61**) and R_f 0.25 (**62**) (3:1 hexanes–EtOAc); **61**: $[\alpha]_D$ –79.8 (*c* 0.9, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 8.09 (dd, 4 H, J = 7.0, 6.8 Hz, Ar), 7.95 (d,

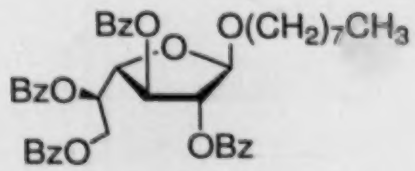
2 H, J = 8.0 Hz, Ar), 7.61–7.29 (m, 11 H, Ar), 7.11 (d, 2 H, J = 8.0 Hz, Ar), 5.76 (d, 1 H, J = 0.9 Hz, H-1), 5.69–5.65 (m, 3 H, H-2, H-3, H-5), 4.92 (dd, 1 H, J = 4.7, 4.7 Hz, H-4), 4.07–4.04 (m, 2 H, H-6_a, H-6_b), 2.33 (s, 3 H, ArCH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 166.5 (C=O), 166.0 (C=O), 165.3 (C=O), 138.3 (Ar), 133.7 (Ar), 133.6 (Ar), 133.1 (Ar), 130.0 (Ar), 129.9 (Ar), 129.9 (Ar), 129.8 (Ar), 129.7 (Ar), 129.4 (Ar), 129.0 (Ar), 128.9 (Ar), 128.6 (Ar), 128.5 (Ar), 128.4 (Ar), 91.9 (C-1), 83.1 (C-4), 81.8 (C-2), 78.2 (C-3), 73.3 (C-5), 62.6 (C-6), 21.1 (ArCH_3). ESI-MS m/z calcd. for (M + Na) $C_{34}H_{30}O_8SNa$: 621.1556. Found: 621.1554. **62**: $[\alpha]_D$ –5.3 (c 0.9, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 8.11 (dd, 2 H, J = 6.5, 2.0 Hz, Ar), 8.04 (dd, 4 H, J = 13.0, 6.5 Hz, Ar), 7.64–7.37 (m, 11 H, Ar), 7.12 (d, 2 H, J = 7.5 Hz, Ar), 5.74 (d, 1 H, J = 1.8 Hz, H-1), 5.73–5.72 (m, 2 H, H-2, H-3), 4.65 (dd, 1 H, J = 5.0, 2.5 Hz, H-4), 4.59–4.48 (m, 3 H, H-6_a, H-5, H-6_b), 2.33 (s, 3 H, ArCH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 166.5 (C=O), 166.0 (C=O), 165.3 (C=O), 138.3 (Ar), 133.7 (Ar), 133.5 (Ar), 133.3 (Ar), 130.0 (Ar), 130.0 (Ar), 129.89 (Ar), 129.87 (Ar), 129.78 (Ar), 129.73 (Ar), 129.4 (Ar), 128.95 (Ar), 128.90 (Ar), 128.58 (Ar), 128.55 (Ar), 128.4 (Ar), 92.0 (C-1), 83.2 (C-2), 81.8 (C-4), 78.2 (C-3), 69.0 (C-5), 66.1 (C-6), 21.1 (ArCH_3). ESI-MS m/z calcd. for (M + Na) $C_{34}H_{30}O_8SNa$: 621.1556. Found: 621.1554.



67

Methyl 2,3,5,-tri-o-benzoyl-β-L-arabinofuranoside (67). L-arabinose (10.0 g, 55.6 mmol) was dissolved in absolute MeOH (200 mL) and to the solution was added 1.06 M methanolic hydrogen chloride (63 mL). The reaction was stirred at rt for 4 h and then neutralized by the addition of pyridine (40 mL). The solvent was evaporated and the syrupy residue was then taken up in pyridine (75 mL). After cooling to 0 °C in an ice bath, benzoyl chloride (31 mL, 2.7 mmol) was added dropwise. The reaction mixture was then allowed to stir overnight while warming to rt. Ice-water was added to destroy the excess benzoyl chloride. The reaction mixture was diluted with CH_2Cl_2 and the layers separated. The organic layer was then washed successively with water, 1M HCl and a saturated aqueous solution of $NaHCO_3$. The organic solution was dried and the solvent evaporated. The residue was then dissolved in warm absolute ethanol and the product was allowed to crystallize overnight, followed by recrystallization overnight from ethanol. The process gave **67** as white crystals (15.88 g, 60%); m.p. 99–100 °C; R_f 0.34 (2:1 Hexanes–EtOAc); $[\alpha]_D$ –18.6 (*c* 1.1, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$, δ_H) 8.20–8.01 (m, 6 H, Ar), 7.67–7.16 (m, 7 H, Ar), 7.36–7.25 (m, 2 H, Ar), 5.60 (dd, 1 H, J = 4.8, 1.0 Hz, H-3), 5.53 (d, 1 H, J = 1.0 Hz, H-2), 5.20 (s, 1 H, H-1), 4.85 (dd, 1 H, J = 12.0, 4.2 Hz, H-5_a), 4.71 (dd, 1 H, J = 12.0, 4.2 Hz, H-5_b), 4.59 (ddd, 1 H, J = 4.8, 4.2, 4.2 Hz, H-4), 3.38 (s, 3 H, OCH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 166.2 (C=O), 165.8 (C=O), 165.5 (C=O), 133.51 (Ar), 133.49 (Ar), 133.5 (Ar), 130.0 (Ar), 129.9 (Ar), 129.8 (Ar), 129.1 (Ar), 128.51 (Ar), 128.47 (Ar), 128.3 (Ar), 106.9 (C-1), 82.2 (C-2), 80.9 (C-

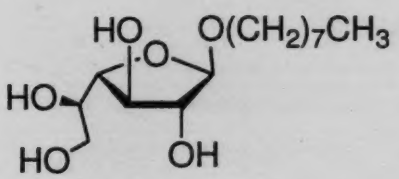
4), 78.0 (C-3), 63.7 (C-5), 55.0 (OCH_3). ESI-MS *m/z* calcd. for (M + Na) $C_{27}H_{24}O_8Na$: 499.1369. Found: 499.1430.



70

Octyl-2,3,5,6,-tetra-*O*-benzoyl-β-D-galactofuranoside (70). Compound **43** (1.01 g, 1.43 mmol), 1-octanol (0.27 mL, 1.70 mmol), and activated, powdered 4 Å molecular sieves 2.5 g in CH_2Cl_2 (60 mL) were cooled at 0 °C. The reaction mixture was stirred for 15 min before *N*-iodosuccinimide (0.48 g, 2.15 mmol), and then AgOTf (0.074 g, 0.29 mmol) were added. The solution was stirred for a further 30 min before the reaction was quenched by the addition of Et_3N. The reaction mixture was then diluted with CH_2Cl_2 and filtered through a pad of Celite. The filtrate was washed with a saturated aqueous solution of $Na_2S_2O_3$, water and brine. The organic layer was then dried and the solvent evaporated. The crude product was purified by column chromatography (4:1 hexanes–EtOAc to afford **70** (0.90 g, 89%). R_f 0.40 (4:1 hexanes–EtOAc); $[\alpha]_D$ –10.6 (*c* 1.5, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 8.05 (dd, 4 H, *J* = 15.0, 6.5 Hz, Ar), 7.98 (dd, 2 H, *J* = 6.5, 3.0 Hz, Ar), 7.85 (dd, 2 H, *J* = 6.5, 3.0 Hz, Ar), 7.59–7.49 (m, 4 H, Ar), 7.44–7.29 (m, 8 H, Ar), 6.10 (ddd, 1 H, *J* = 5.8, 5.8, 4.0 Hz, H-5), 5.66 (d, 1 H, *J* = 5.0 Hz, H-3), 5.49 (d, 1 H, *J* = 1.2 Hz, H-2), 5.32 (br s, 1 H, H-1), 4.81 (dd, 1 H, *J* = 12.0, 5.8 Hz, H-6_a), 4.78 (dd, 1 H, *J* = 12.0, 5.8 Hz, H-6_b), 4.65 (dd, 1 H, *J* = 5.0, 4.0 Hz, H-4), 3.75 (ddd, 1 H, *J* = 9.6, 6.5, 6.5 Hz, octyl OCH_2), 3.53 (ddd, 1 H, *J* = 9.6, 6.5, 6.5 Hz, octyl OCH_2), 1.64–1.61 (m, 2 H, octyl CH_2), 1.36–1.29 (m, 10 H, octyl CH_2), 0.88 (t, 3 H, *J* =

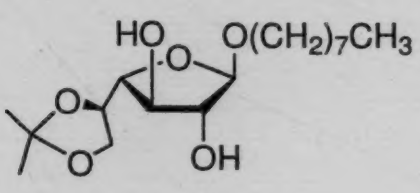
7.0 Hz, octyl CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 166.1 (C=O), 165.8 (C=O), 165.7 (C=O), 165.5 (C=O), 133.4 (Ar), 133.3 (Ar), 133.2 (Ar), 133.1 (Ar), 130.0 (Ar), 130.0 (Ar), 129.8 (Ar), 129.7 (Ar), 129.6 (Ar), 129.6 (Ar), 129.1 (Ar), 129.0 (Ar), 128.4 (Ar), 128.4 (Ar), 105.6 (C-1), 82.1 (C-2), 81.3 (C-4), 77.6 (C-3), 70.4 (C-5), 67.7 (octyl OCH_2), 63.5 (C-6), 31.8 (octyl CH_2), 29.5 (octyl CH_2), 29.4 (octyl CH_2), 29.3 (octyl CH_2), 26.2 (octyl CH_2), 22.7 (octyl CH_2), 14.1 (octyl CH_3). ESI-MS *m/z* calcd. for (M+Na) $C_{42}H_{44}O_{10}Na$: 731.2854. Found: 731.2851.



71

Octyl β-D-galactofuranoside (71). Prepared from **70** (1.47 g, 2.08 mmol) in MeOH (50 mL) and NaOMe in MeOH (0.1 M) as described for **57**. The reaction mixture was concentrated to give a syrupy residue that was purified by column chromatography (9:1 CH_2Cl_2–MeOH) to afford **71** (0.56 g, 92%) as a yellow solid. R_f 0.31 (7:1 CH_2Cl_2–MeOH); $[\alpha]_D$ –10.5 (*c* 1.2, MeOH); ^1H NMR (500 MHz, CD_3OD, δ_H) 4.83 (d, 1 H, *J* = 1.5 Hz, H-1), 3.98 (dd, 1 H, *J* = 5.0, 4.0 Hz, H-3), 3.92 (dd, 1 H, *J* = 4.0, 1.5 Hz, H-2), 3.89 (dd, 1 H, *J* = 6.5, 5.0 Hz, H-4), 3.74–3.69 (m, 2 H, H-5, octyl OCH_2), 3.64–3.60 (m, 2 H, H-6_a, H-6_b), 3.40 (ddd, 1 H, *J* = 9.6, 6.5, 6.5 Hz, octyl OCH_2), 1.60–1.52 (m, 2 H, octyl CH_2), 1.38–1.22 (m, 10 H, octyl CH_2), 0.90 (t, 3 H, *J* = 7.0 Hz, octyl CH_3); ^{13}C NMR (125 MHz, CD_3OD, δ_C) 109.4 (C-1), 84.2 (C-2), 83.5 (C-4), 78.8 (C-3), 72.5 (C-5), 70.5 (octyl OCH_2), 65.0 (C-6), 33.0 (octyl CH_2), 30.7 (octyl CH_2), 30.5 (octyl CH_2), 30.4

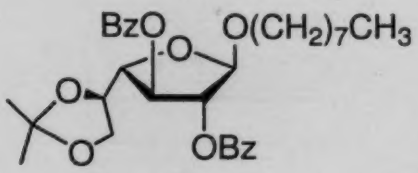
(octyl CH_2), 27.3 (octyl CH_2), 23.7 (octyl CH_2), 14.4 (octyl CH_3). ESI-MS *m/z* calcd. for (M+Na) $C_{14}H_{28}O_6Na$: 315.1784. Found: 315.1834.



72

Octyl 5,6-isopropyledine-β-D-galactofuranoside (72). To a solution of **71** (0.25 g, 0.86 mmol) in acetone (5 mL) was added 2,2-dimethoxypropane (0.16 mL, 1.29 mmol) and (1*S*)-(+)-camphorsulfonic acid (0.02 g, 0.09 mmol) at rt. After 3 h of stirring, the pH of the solution was adjusted to pH 7 by adding Et_3N. The reaction mixture was concentrated to give a residue that was subsequently dissolved in CH_2Cl_2 (10 mL), washed with a saturated aqueous solution of $NaHCO_3$ and water before being dried and concentrated to an oily residue, which was purified by column chromatography (5:1 hexanes–EtOAc) to give **72** (0.27 g, 94%) as a colorless oil. R_f 0.40 (1:1 hexanes–EtOAc); $[\alpha]_D$ –26.8 (*c* 1.2, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$, δ_H) 4.98 (s, 1 H, H-1), 4.28 (ddd, 1 H, J = 7.5, 7.5, 1.5 Hz, H-5), 4.03 (dd, 1 H, J = 7.5, 6.8 Hz, H-4), 4.03 (dd, 1 H, J = 6.8, 1.5 Hz, H-3), 4.08–3.90 (m, 4 H, H-2, H-6_a, H-6_b, 3-OH), 3.68 (ddd, 1 H, J = 9.6, 6.8, 6.8 Hz, octyl OCH_2), 3.40 (ddd, 1 H, J = 9.6, 6.8, 6.8 Hz, octyl OCH_2), 3.03 (d, 1 H, 2-OH), 1.64–1.61 (m, 2 H, octyl CH_2), 1.39 (s, 3 H, isopropylidene CH_3), 1.35 (s, 3 H, isopropylidene CH_3), 1.32–1.19 (m, 10 H, octyl CH_2), 0.84 (t, 3 H, J = 7.0 Hz, octyl CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 110.1 ($(CH_3)_2C$), 108.4 (C-1), 85.5 (C-2), 78.6 (C-4), 78.0 (C-3), 75.7 (C-5), 67.8 (octyl OCH_2), 65.7 (C-6), 31.7 (octyl CH_2), 29.4 (octyl

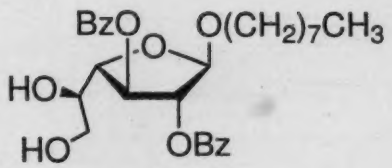
CH_2), 29.2 (octyl CH_2), 29.1 (octyl CH_2), 26.0 (octyl CH_2), 25.6 (isopropylidene CH_3), 25.5 (isopropylidene CH_3), 22.6 (octyl CH_2), 14.0 (octyl CH_3). ESI-MS *m/z* calcd. for (M+Na) $C_{17}H_{32}O_6Na$: 355.2097. Found: 355.2137.



74

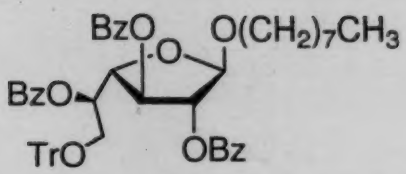
Octyl 5,6-isopropylidine-2,3-di-*O*-benzoyl-β-D-galactofuranoside (74). Prepared from **72** (1.00 g, 2.00 mmol) in pyridine (15 mL) and benzoyl chloride (0.06 mL, 5.0 mmol) as described for **57**. The crude product was purified by column chromatography (3:1 hexanes–EtOAc) to give **74** (1.48 g, 91%) as a colorless oil. R_f 0.34 (4:1 hexanes–EtOAc); $[\alpha]_D$ –30.3 (*c* 1.1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 8.08–8.01 (m, 4 H, Ar), 7.62–7.58 (m, 2 H, Ar), 7.46–7.38 (m, 4 H, Ar), 5.44 (d, 1 H, J = 4.5 Hz, H-3), 5.42 (d, 1 H, J = 1.1 Hz, H-2), 5.26 (br s, 1 H, H-1), 4.50 (ddd, 1 H, J = 6.5, 6.5, 4.0 Hz, H-5), 4.29 (dd, 1 H, J = 4.5, 4.5 Hz, H-4), 4.14 (dd, 1 H, J = 10.0, 6.5 Hz, H-6_a), 3.97 (dd, 1 H, J = 10.0, 6.5 Hz, H-6_b), 3.78 (ddd, 1 H, J = 9.5, 6.8, 6.8 Hz, octyl OCH_2), 3.41 (ddd, 1 H, J = 9.5, 6.8, 6.8 Hz, octyl OCH_2), 1.63–1.58 (m, 2 H, octyl CH_2), 1.45 (s, 3 H, isopropylidene CH_3), 1.40 (s, 3 H, isopropylidene CH_3), 1.25–1.21 (m, 10 H, octyl CH_2), 0.86 (t, 3 H, J = 7.1 Hz, octyl CH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 165.6 (C=O), 165.3 (C=O), 130.5 (Ar), 129.92 (Ar), 129.89 (Ar), 129.3 (Ar), 129.28 (Ar), 128.4 (Ar), 110.0 ($(CH_3)_2C$), 105.7 (C-1), 83.3 (C-2), 81.7 (C-4), 77.7 (C-3), 75.8 (C-5), 67.6 (octyl OCH_2), 65.7 (C-6), 31.8 (octyl CH_2), 29.6 (octyl CH_2), 29.4 (octyl CH_2), 29.3 (octyl CH_2), 26.4 (octyl CH_2), 22.7 (octyl CH_2), 26.2 (isopropylidene CH_3), 25.3 (isopropylidene CH_3),

14.1 (octyl CH_3). ESI-MS *m/z* calcd. for (M+Na) $C_{31}H_{40}O_8Na$: 563.2649. Found:563.2650.



75

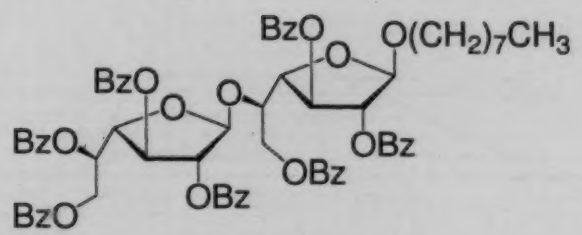
Octyl 2,3-di-*O*-benzoyl-β-D-galactofuranoside (75). To a solution of **74** (1.75 g, 3.24 mmol) in CH_2Cl_2 (25 mL) was added trifluoroacetic acid containing 1% H_2O (5 mL). The reaction mixture was stirred for 2 h and then ethanol (10 mL) was added. The solution was then concentrated and then coevaporated with toluene. The resulting residue was purified by chromatography (2:1 hexanes–EtOAc) to give **75** (1.46 g, 90%) as a colorless oil. R_f 0.25 (2:1 hexanes–EtOAc); $[\alpha]_D$ –15 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 8.07 (d, 4 H, *J* = 7.6 Hz, Ar), 7.60–7.54 (m, 2 H, Ar), 7.44 (dd, 4 H, *J* = 7.6, 7.6 Hz, Ar), 5.59 (dd, 1 H, *J* = 4.5, 0.5 Hz, H-3), 5.49 (br s, 1 H, H-2), 5.25 (s, 1 H, H-1), 4.29 (dd, 1 H, *J* = 4.5, 4.5 Hz, H-4), 4.14 (ddd, 1 H, *J* = 5.1, 5.1, 4.5 Hz, H-5), 3.86 (dd, 1 H, *J* = 11.8, 5.1 Hz, H-6_a), 3.80 (dd, 1 H, *J* = 11.8, 5.1 Hz, H-6_b), 3.75 (ddd, 1 H, *J* = 9.5, 6.7, 6.7 Hz, octyl OCH_2), 3.53 (ddd, 1 H, *J* = 9.5, 6.7. 6.7 Hz, octyl OCH_2), 1.63–1.59 (m, 2 H, octyl CH_2), 1.33–1.27 (m, 10 H, octyl CH_2), 0.86 (t, 3 H, *J* = 7.2 Hz, octyl CH_3); ^{13}C NMR (75 MHz, $CDCl_3$, δ_C) 165.1 (C=O), 165.4 (C=O), 133.5 (Ar), 129.92 (Ar), 129.86 (Ar), 129.2 (Ar), 129.1 (Ar), 128.5 (Ar), 128.4 (Ar), 105.7 (C-1), 84.0 (C-2), 81.4 (C-4), 78.0 (C-3), 70.9 (C-5), 67.7 (octyl OCH_2), 64.3 (C-6), 31.8 (octyl CH_2), 29.5 (octyl CH_2), 29.4 (octyl CH_2), 29.2 (octyl CH_2), 26.1 (octyl CH_2), 22.6 (octyl CH_2), 14.1 (octyl CH_3). ESI-MS *m/z* calcd. for (M+Na) $C_{28}H_{36}O_8Na$: 523.2344. Found: 523.2340.



76

Octyl 2,3,5-tri-*O*-benzoyl-6-*O*-trityl-β-D-galactofuranoside (76). Prepared through tritylation of **71** as described for **59** (0.11 g, 0.14 mmol), in pyridine (5 mL), trityl chloride (0.06 g, 0.21 mmol), DMAP (3.40 mg, 0.03 mmol); and then benzoylation as described for **57**, benzoyl chloride (23 μL, 0.20 mmol). The resulting residue was purified by column chromatography (4:1 hexanes–EtOAc) to afford **76** (0.11 g, 87%) as a white solid. R_f 0.42 (4:1 hexanes–EtOAc); $[\alpha]_D$ –1.0 (*c* 1.1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 8.18 (d, 2 H, *J* = 5.0 Hz, Ar), 8.06 (d, 2 H, *J* = 7.0 Hz, Ar), 7.90 (d, 2 H, *J* = 7.0 Hz, Ar), 7.62–7.16 (m, 24 H, Ar), 5.80 (ddd, 1 H, *J* = 5.7, 5.7, 4.2 Hz, H-5), 5.50 (dd, 1 H, *J* = 5.1, 1.2 Hz, H-3), 5.37 (dd, 1 H, *J* = 1.2, 0.5 Hz, H-2), 5.23 (br s, 1 H, H-1), 4.76 (dd, 1 H, *J* = 5.1, 4.2 Hz, H-4), 3.71 (ddd, 1 H, *J* = 9.5, 6.8, 6.8 Hz, octyl OCH_2), 3.56 (dd, 1 H, *J* = 9.9, 5.7 Hz, H-6_a), 3.43 (dd, 1 H, *J* = 9.9, 5.7 Hz, H-6_b), 3.52 (ddd 1 H, *J* = 9.5, 6.8, 6.8 Hz, octyl OCH_2), 1.63–1.59 (m, 2 H, octyl CH_2), 1.28–1.20 (m, 10 H, octyl CH_2), 0.88 (t, 3 H, *J* = 7.1 Hz, octyl CH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ_H) 165.7 (C=O), 165.50 (C=O), 165.46 (C=O), 143.6 (Ar), 133.5 (Ar), 133.1 (Ar), 130.0 (Ar), 129.98 (Ar), 129.95 (Ar), 129.90 (Ar), 129.85 (Ar), 129.80 (Ar), 129.3 (Ar), 129.2 (Ar), 128.7 (Ar), 128.69 (Ar), 128.61 (Ar), 128.4 (Ar), 128.35 (Ar), 128.32 (Ar), 128.26 (Ar), 127.8 (Ar), 127.71 (Ar), 127.68 (Ar), 127.0 (Ar), 126.9 (Ar), 105.4 (C-1), 86.9 (Ph_3C), 82.3 (C-2), 81.0 (C-4), 77.7 (C-3), 72.1 (C-5), 67.5 (octyl OCH_2), 62.7 (C-6), 31.8 (octyl CH_2), 29.5 (octyl CH_2), 29.4 (octyl CH_2), 29.3 (octyl CH_2), 26.2 (octyl CH_2), 22.7 (octyl CH_2),

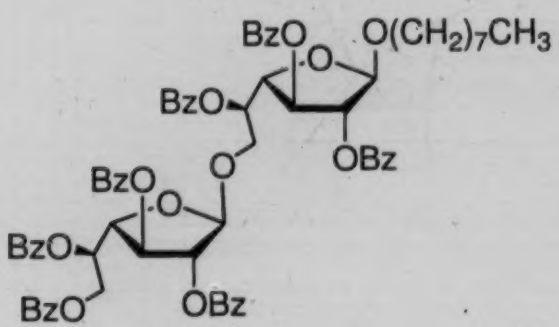
14.1 (octyl CH_3). ESI-MS *m/z* calcd. for (M+Na) $C_{54}H_{54}O_9$: 869.3662. Found: 869.3661.



77

Octyl 2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl)-(1→5)-2,3,6-tri-*O*-benzoyl-β-D-galactofuranoside (77). Prepared from **40** (0.05 g, 0.08 mmol), donor **43** (0.06 g, 0.09 mmol), NIS (0.03 g, 0.12 mmol), and AgOTf (4.0 mg, 0.02 mmol) in CH_2Cl_2 (3 mL) as described for **70**. The residue was purified by column chromatography (4:1 hexanes–EtOAc) to afford **77** (0.24 g, 87%) as a colorless oil. R_f 0.37 (5:1 hexanes–EtOAc); $[\alpha]_D$ −10.4 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 8.02–7.93 (m, 8 H, Ar), 7.91–7.75 (m, 4 H, Ar), 7.62–7.52 (m, 7 H, Ar), 7.48–7.39 (m, 16 H, Ar), 6.08 (ddd, 1 H, *J* = 7.0, 7.0, 3.6 Hz, H-5'), 5.84 (dd, 1 H, *J* = 5.0, 1.4 Hz, H-3), 5.81 (s, 1 H, H-1'), 5.69 (d, 1 H, *J* = 1.2 Hz, H-2'), 5.63 (dd, 1 H, *J* = 5.1, 1.2 Hz, H-3'), 5.49 (dd, 1 H, *J* = 1.4, 0.6 Hz, H-2), 5.21 (br s, 1 H, H-1), 5.06 (dd, 1 H, *J* = 5.1, 3.6 Hz, H-4'), 4.79 (m, 5 H, H-6_a, H-6_a', H-5, H-6_b, H-6_b'), 4.53 (dd, 1 H, *J* = 5.0, 1.1 Hz, H-4), 3.72 (ddd, 1 H, *J* = 10.0, 6.0, 6.0 Hz, octyl OCH_2), 3.47 (ddd, 1 H, *J* = 10.0, 6.0, 6.0 Hz, octyl OCH_2), 1.64–1.61 (m, 2 H, octyl CH_2), 1.36–1.29 (m, 10 H, octyl CH_2), 0.88 (t, 3 H, *J* = 7.0 Hz, octyl CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 166.1 (C=O), 166.0 (C=O), 165.7 (C=O), 165.7 (C=O), 165.5 (C=O), 165.4 (C=O), 165.2 (C=O), 133.38 (Ar), 133.36 (Ar), 133.24 (Ar), 133.20 (Ar), 133.1 (Ar), 133.04 (Ar), 133.02 (Ar), 133.0 (Ar), 132.9 (Ar), 130.0 (Ar), 129.93

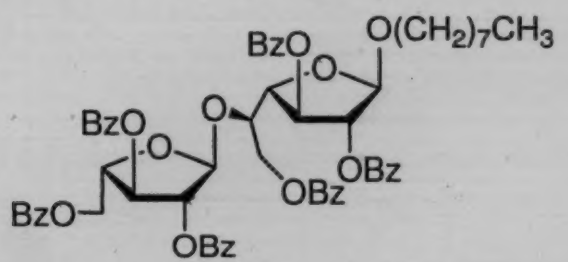
(Ar), 129.90 (Ar), 129.8 (Ar), 129.75 (Ar), 129.70 (Ar), 129.6 (Ar), 129.0 (Ar), 128.90 (Ar), 128.87 (Ar) 128.5 (Ar), 128.4 (Ar), 128.3 (Ar), 128.2 (Ar), 128.1 (Ar), 105.5 (C-1'), 105.2 (C-1), 82.4 (C-2'), 82.1 (C-2), 82.0 (C-4'), 81.7 (C-4), 80.6 (C-3), 77.9 (C-3'), 77.3 (C-5), 77.1 (C-5'), 69.5 (C-6'), 67.8 (octyl OCH_2), 63.0 (C-6), 31.9 (octyl CH_2), 29.6 (octyl CH_2), 29.4 (octyl CH_2), 29.3 (octyl CH_2), 26.1 (octyl CH_2), 22.7 (octyl CH_2), 14.11 (octyl CH_3). ESI-MS *m/z* calcd. for (M + Na) $C_{69}H_{66}O_{18}Na$: 1205.4123. Found: 1205.4115.



78

Octyl 2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl-(1→6)-2,3,5-tri-O-benzoyl-β-D-galactofuranoside (78). Prepared from **39** (0.05 g, 0.08 mmol) and donor **43** (0.06 g, 0.09 mmol), NIS (0.03 g, 0.12 mmol), and AgOTf (4.0 mg, 0.02 mmol) in CH_2Cl_2 (2 mL) as described for **70**. The residue was purified by column chromatography (4:1 hexanes–EtOAc) to give **78** (0.08 g, 89%) as a colorless oil. R_f 0.37 (5:1 hexanes–EtOAc); $[\alpha]_D$ –2.3 (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$, δ_H) 8.20–7.99 (m, 8 H, Ar), 7.97–7.90 (m, 4 H, Ar), 7.80 (dd, 2 H, *J* = 7.0, 2.0 Hz, Ar), 7.50–7.41 (m, 8 H, Ar), 7.35–7.26 (m, 8 H, Ar), 7.25–7.20 (m, 5 H, Ar), 6.08 (ddd, 1 H, *J* = 5.0, 5.0, 3.6 Hz, H-5'), 5.92 (ddd, 1 H, *J* = 5.0, 5.0, 3.9 Hz, H-5), 5.64-5.58 (m, 3 H, H-3, H-3', H-2), 5.45 (br s, 1 H, H-1'),

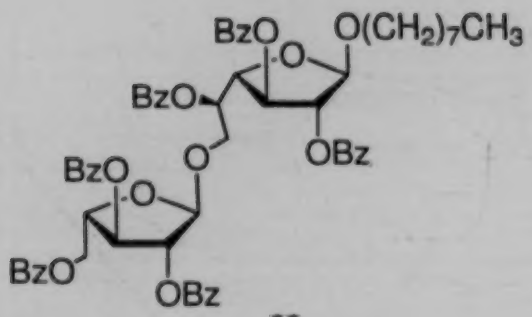
5.41 (dd, 1 H, J = 5.0, 1.0 Hz, H-2'), 5.28 (s, 1 H, H-1), 4.78 (dd, 1 H, J = 5.0, 3.4 Hz, H-4), 4.76–4.73 (m, 2 H, H-6_a', H-6_b'), 4.70 (dd, 1 H, J = 5.0, 3.6 Hz, H-4'), 4.23 (dd, 1 H, J = 10.5, 5.0 Hz, H-6_a), 4.06 (dd, 1 H, J = 10.5, 5.0 Hz, H-6_b), 3.75 (ddd, 1 H, J = 9.5, 7.0, 7.0 Hz, octyl OCH_2), 3.52 (ddd, 1 H, J = 9.5, 7.0, 7.0 Hz, octyl OCH_2), 1.63–1.40 (m, 2 H, octyl CH_2), 1.28–1.40 (m, 10 H, octyl CH_2), 0.89 (t, 3 H, J = 7.5, octyl CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 166.1 (C=O), 165.8 (C=O), 165.69 (C=O), 165.67 (C=O), 165.5 (C=O), 165.4 (C=O), 165.2 (C=O), 133.38 (Ar), 133.36, 133.2 (Ar), 133.19 (Ar), 133.1 (Ar), 133.07 (Ar), 133.04 (Ar), 133.02 (Ar), 132.97 (Ar), 132.9 (Ar), 130.0 (Ar), 129.93 (Ar), 129.90 (Ar), 129.8 (Ar), 129.75 (Ar), 129.70 (Ar), 129.6 (Ar), 129.0 (Ar), 128.89 (Ar), 129.87 (Ar), 128.5 (Ar), 128.4 (Ar), 128.3 (Ar), 128.2 (Ar), 128.1 (Ar), 106.5 (C-1'), 105.8 (C-1), 82.2 (C-2'), 81.4 (C-2), 81.8 (C-4'), 81.4 (C-4), 77.9 (C-3), 77.32 (C-3'), 77.30 (C-5), 77.1 (C-5'), 71.2 (C-6), 67.9 (octyl OCH_2), 63.7 (C-6'), 31.9 (octyl CH_2), 29.6 (octyl CH_2), 29.4 (octyl CH_2), 29.3 (octyl CH_2), 26.2 (octyl CH_2), 22.7 (octyl CH_2), 14.1 (octyl CH_3). ESI-MS m/z calcd. for (M + Na) $C_{69}H_{66}O_{18}Na$: 1205.4123. Found: 1205.4117.



79

Octyl 2,3,5,-tri-*O*-benzoyl-β-L-arabinofuranosyl-(1→5)-2,3,6-tri-*O*-benzoyl-β-D-galactofuranoside (79). Prepared from **40** (35.3 mg, 0.06 mmol), donor **44** (40.8 mg,

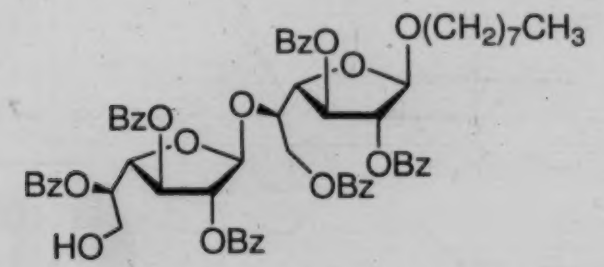
0.08 mmoL), NIS (19.7 mg, 0.09 mmol), and AgOTf (3.0 mg, 0.01 mmol) in CH_2Cl_2 (3 mL) as described for **70**. The residue was then purified by chromatography (6:1 hexanes–EtOAc) to give disaccharide **79** (53.0 mg, 87%) as a colorless oil. R_f 0.45 (3:1 hexanes–EtOAc); $[\alpha]_D$ –10.9 (*c* 0.8, $CHCl_3$); ^{1}H NMR (400 MHz,$CDCl_3$, δ_H) 8.05–7.90 (m, 12 H, Ar), 7.61–7.21 (m, 18 H, Ar), 5.82 (dd, 1 H, *J* = 4.2, 1.7 Hz, H-3), 5.73 (s, 1 H, H-1'), 5.70 (d, 1 H, *J* = 1.4 Hz, H-2'), 5.58 (dd, 1 H, *J* = 5.0, 1.4 Hz, H-3'), 5.48 (d, 1 H, *J* = 1.7 Hz, H-2), 5.22 (s, 1 H, H-1), 4.82 (ddd, 1 H, *J* = 5.0, 4.5, 4.5 Hz, H-4'), 4.78–4.64 (m, 4 H, H-5_a', H-6_b, H-6_a, H-5), 4.58–4.52 (m, 2 H, H-5_b', H-4), 3.75 (ddd, 1 H, *J* = 9.5, 7.0, 7.0 Hz, octyl OCH_2), 3.52 (ddd, 1 H, *J* = 9.5, 7.0, 7.0 Hz, octyl OCH_2), 1.63–1.59 (m, 2 H, octyl CH_2), 1.38–1.20 (m, 10 H, octyl CH_2), 0.89 (t, 3 H, *J* = 7.5 Hz, octyl CH_3); ^{13}C NMR (100 MHz,$CDCl_3$, δ_C) 166.2 (C=O),166.1 (C=O),165.6 (C=O), 165.5 (C=O), 165.4 (C=O), 165.2 (C=O),133.5 (Ar), 133.41 (Ar), 133.38 (Ar), 133.36 (Ar), 133.31 (Ar), 132.95 (Ar), 132.90 (Ar), 129.90 (Ar), 129.87 (Ar), 129.84 (Ar), 129.77 (Ar), 129.71 (Ar), 129.6 (Ar), 129.1 (Ar), 129.0 (Ar), 128.9 (Ar), 128.5 (Ar), 128.4 (Ar), 128.36 (Ar), 128.3 (Ar), 128.27 (Ar), 128.2 (Ar), 106.0 (C-1), 105.6 (C-1'), 82.3 (C-2), 82.0 (C-2'), 81.9 (C-4), 81.3 (C-4'), 77.8 (C-3), 77.4 (C-3'), 74.0 (C-5), 67.7 (C-5'), 64.7 (octyl OCH_2), 63.6 (C-6), 31.9 (octyl CH_2), 29.6 (octyl CH_2), 29.4 (octyl CH_2), 29.3 (octyl CH_2), 26.2 (octyl CH_2), 22.7 (octyl CH_2), 14.1 (octyl CH_3). ESI-MS *m/z* calcd. for (M + Na) $C_{61}H_{60}O_{16}Na$: 1071.3883. Found: 1071.3773.



80

Octyl 2,3,5,-tri-*O*-benzoyl-β-L-arabinofuranosyl-(1→6)-2,3,6-tri-*O*-benzoyl-β-D-galactofuranoside (80). Prepared from **40** (52.9 mg, 0.09 mmol), donor **44** (61.2 mg, 0.11 mmol), NIS (29.5 mg, 0.13 mmol) and AgOTf (4.5 mg, 0.02 mmol) in CH_2Cl_2 (3 mL) as described for **70**. The residue was then purified by chromatography (6:1 hexanes–EtOAc) to give disaccharide **80** (82.0 mg, 89%) as colorless oil. R_f 0.43 (3:1 hexanes–EtOAc); $[\alpha]_D$ –20.3 (*c* 1.1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 8.17–8.01 (m, 8 H, Ar), 7.97–7.81 (m, 4 H, Ar), 7.60–7.22 (m, 18 H, Ar), 5.89 (ddd, 1 H, *J* = 6.5, 6.5, 5.0 Hz, H-5), 5.63 (d, 1 H, *J* = 6.0 Hz, H-3), 5.56 (d, 1 H, *J* = 4.0 Hz, H-3'), 5.47–5.42 (m, 2 H, H-2, H-2'), 5.35 (s, 1 H, H-1'), 5.29 (s, 1 H, H-1), 4.71–4.61 (m, 4 H, H-5$_a$', H-5$_b$', H-4, H-4'), 4.23 (dd, 1 H, *J* = 11.0, 5.0 Hz, H-6$_a$), 4.08 (dd, 1 H, *J* = 11.0, 5.0 Hz, H-6$_b$), 3.75 (ddd, 1 H, *J* = 9.5, 7.0, 7.0 Hz, octyl OCH_2), 3.52 (ddd, 1 H, *J* = 9.5, 7.0, 7.0 Hz, octyl OCH_2), 1.63–1.59 (m, 2 H, octyl CH_2), 1.38–1.20 (m, 10 H, octyl CH_2), 0.89 (t, 3 H, *J* = 7.5 Hz, octyl CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 166.1 (C=O), 165.8 (C=O), 165.7 (C=O), 165.5 (C=O), 165.1 (C=O), 165.2 (C=O), 133.4 (Ar), 133.4 (Ar), 133.3 (Ar), 133.2 (Ar), 133.1 (Ar), 133.0 (Ar), 132.98 (Ar), 132.95 (Ar), 130.0 (Ar), 129.9 (Ar), 129.89 (Ar), 129.83 (Ar), 129.80 (Ar), 129.7 (Ar), 129.2 (Ar), 129.0 (Ar), 128.9 (Ar), 128.5 (Ar), 128.40 (Ar), 128.37 (Ar), 128.35 (Ar), 128.31 (Ar), 128.2 (Ar), 106.1 (C-1), 105.7 (C-1'), 82.2 (C-2), 82.0 (C-2'), 81.4 (C-4), 81.2 (C-4'), 77.7 (C-3), 77.4 (C-

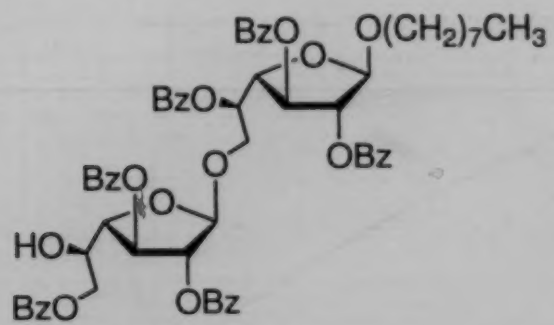
3'), 71.4 (C-5), 67.8. (C-6'), 66.1 (octyl OCH_2), 63.5 (C-5'), 31.9 (octyl CH_2), 29.6 (octyl CH_2), 29.4 (octyl CH_2), 29.3 (octyl CH_2), 26.2 (octyl CH_2), 22.7 octyl (CH_2), 14.1 (octyl CH_3). ESI-MS *m/z* calcd. for (M + Na) $C_{61}H_{60}O_{16}Na$: 1071.3776. Found: 1071.3773.



81

Octyl 2,3,5-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→5)-2,3,6-tri-*O*-benzoyl-β-D-galactofuranoside (81). Compound **83** (0.23 g, 0.21 mmol) was dissolved in CH_2Cl_2 (5 mL) and 3% methanolic HCl (12.0 mL) and the reaction mixture was stirred for 8 h. The solution was partially concentrated, diluted with CH_2Cl_2 and was washed with saturated $NaHCO_3$, water, and brine. The organic layer was then dried and the solvent was concentrated to a residue that was purified by column chromatography (4:1 hexanes–EtOAc) to give disaccharide **81** (0.17 g, 75%) as a colorless oil. R_f 0.26 (3:1 hexanes–EtOAc); $[\alpha]_D$ –20.8 (*c* 2.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 8.20–7.90 (m, 8 H, Ar), 7.88–7.82 (m, 4 H, Ar), 7.59–7.42 (m, 8 H, Ar), 7.36–7.24 (m, 10 H, Ar), 5.83 (dd, 1 H, *J* = 5.3, 1.1 Hz, H-3), 5.79 (br s, 1 H, H-1'), 5.69 (dd, 1 H, *J* = 1.7, 0.5 Hz, H-2'), 5.58 (dd, 1 H, *J* = 4.2, 1.7 Hz, H-3'), 5.56–5.53 (m, 1 H, H-5'), 5.50 (d, 1 H, *J* = 1.1 Hz, H-2), 5.24 (br s, 1 H, H-1), 5.02 (dd, 1 H, *J* = 6.0, 4.2 Hz, H-4'), 4.78–4.66 (m, 3 H, H-5, H-6_a, H-6_b), 4.52 (dd, 1 H, *J* = 5.3, 3.4 Hz, H-4), 4.06–3.92 (m, 2 H, H-6'$_a$, H-6'$_b$), 3.74 (ddd, 1 H, *J* = 9.0, 7.0, 7.0 Hz, octyl OCH_2), 3.48 (ddd, 1 H, *J* = 9.0, 7.0, 7.0 Hz, octyl OCH_2),

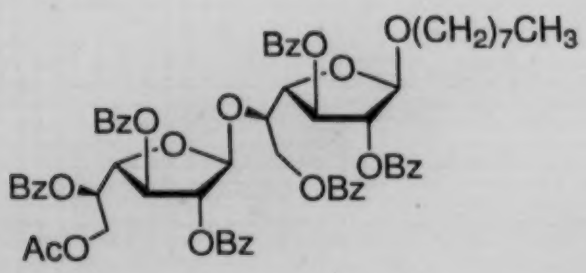
1.63–1.59 (m, 2 H, octyl CH_2), 1.29–1.23 (m, 10 H, octyl CH_2), 0.85 (t, 3 H, J = 9.0 Hz, octyl CH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 166.4 (C=O), 166.1 (C=O), 166.0 (C=O), 165.6 (C=O), 165.5 (C=O), 165.2 (C=O), 133.7 (Ar), 133.4 (Ar), 133.30 (Ar), 133.27 (Ar), 133.1 (Ar), 133.0 (Ar), 130.1 (Ar), 130.0 (Ar), 129.9 (Ar), 129.84 (Ar), 129.81 (Ar), 129.75 (Ar), 129.7 (Ar), 129.6 (Ar),129.0 (Ar), 128.9 (Ar), 128.9 (Ar), 128.8 (Ar), 128.51 (Ar), 128.46 (Ar), 128.35 (Ar), 128.33 (Ar), 128.30 (Ar), 128.2 (Ar), 105.6 (C-1'), 105.5 (C-1), 82.4 (C-2'), 82.1 (C-2), 81.9 (C-4), 81.6 (C-4'), 77.7 (C-3'), 77.5 (C-3), 73.5 (C-5'), 73.0 (C-5), 67.7 (octyl O*C*H_2), 64.8 (C-6), 61.4 (C-6'), 31.8 (octyl *C*H_2), 29.5 (octyl *C*H_2), 29.44 (octyl *C*H_2), 29.3 (octyl *C*H_2), 26.2 (octyl *C*H_2), 22.7 (octyl *C*H_2), 14.2 (octyl *C*H_3). ESI-MS *m/z* calcd. for (M + Na) $C_{62}H_{62}O_{17}$: 1101.3880. Found: 1101.3879.



82

Octyl 2,3,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→6)-2,3,5-tri-*O*-benzoyl-β-D-galactofuranoside (82). Prepared from **86** (0.34 g, 0.06 mmol) in CH_2Cl_2 (20 mL) and 3% methanolic HCl (17.35 mL) as described for **81**. The residue was purified by column chromatography (4:1 hexanes–EtOAc) to give disaccharide **82** (0.28 g, 85%) as a colorless oil. R_f 0.29 (2:1 hexanes–EtOAc); $[\alpha]_D$ –7.1 (*c* 0.3, $CHCl_3$); ^{1}H NMR (500

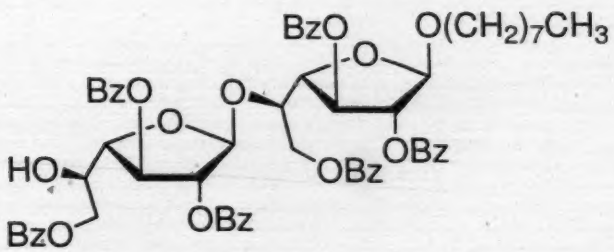
MHz, $CDCl_3$, δ_H) 8.20–7.90 (m, 8 H, Ar), 7.88–7.82 (m, 4 H, Ar), 7.58–7.22 (m, 18 H, Ar), 5.90 (ddd, 1 H, J = 5.9, 5.9, 4.5 Hz, H-5), 5.61 (dd, 1 H, J = 3.7, 1.1 Hz, H-3'), 5.88 (dd, 1 H, J = 3.8, 0.7 Hz, H-3), 5.44 (d, 1 H, J = 1.1 Hz, H-2'), 5.42 (d, 1 H, J = 0.7 Hz, H-2), 5.35 (s, 1 H, H-1'), 5.26 (s, 1 H, H-1), 4.65 (dd, 1 H, J = 4.5, 3.8 Hz, H-4), 4.59 (dd, 1 H, J = 11.6, 6.9 Hz, H-6_a'), 4.52 (dd, 1 H, J = 4.8, 3.7 Hz, H-4'), 4.48–4.02 (m, 2 H, H-5', H-6_b'), 4.16 (dd, 1 H, J = 10.8, 5.9 Hz, H-6_a), 4.05 (dd, 1 H, J = 10.8, 5.9 Hz, H-6_b), 3.72 (ddd, 1 H, J = 9.4, 6.7, 6.7 Hz, octyl OCH_2), 3.50 (ddd, 1 H, J = 9.4, 6.7, 6.7 Hz, octyl OCH_2), 1.63–1.60 (m, 2 H, octyl CH_2), 1.28–1.20 (m, 10 H, octyl CH_2), 0.89 (t, 3 H, J = 7.0 Hz, octyl CH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 166.1 (C=O), 165.8 (C=O), 165.7 (C=O), 165.6 (C=O), 165.5 (C=O), 165.1 (C=O), 133.5 (Ar), 133.4 (Ar), 133.3 (Ar), 133.3 (Ar), 133.03 (Ar), 133.0 (Ar), 129.9 (Ar), 129.83 (Ar), 129.79 (Ar), 129.71 (Ar), 129.2 (Ar), 129.1 (Ar), 129.0 (Ar), 128.7 (Ar), 128.5 (Ar), 128.44 (Ar), 128.44 (Ar), 128.3 (Ar), 127.7 (Ar), 126.9 (Ar), 106.4 (C-1), 105.7 (C-1'), 82.6 (C-2), 82.1 (C-2'), 81.5 (C-4), 81.4 (C-4'), 78.0 (C-3'), 77.5 (C-3), 71.4 (C-5), 69.2 (C-5'), 66.4 (octyl OCH_2), 66.3 (C-6), 61.4 (C-6'), 31.9 (octyl CH_2), 29.6 (octyl CH_2), 29.4 (octyl CH_2), 29.3 (octyl CH_2), 26.2 (octyl CH_2), 22.7 (octyl CH_2), 14.1 (octyl CH_3). ESI-MS *m/z* calcd. for (M + Na) $C_{62}H_{62}O_{17}$: 1101.3881. Found: 1101.3879.



83

Octyl 6-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→5)-2,3,6-tri-*O*-benzoyl-β-D-galactofuranoside (83). Prepared from **40** (0.19 g, 0.31 mmol), donor **42** (0.16 g, 0.25 mmol), NIS (67.7 mg, 0.30 mmol) and AgOTf (12.9 mg, 0.05 mmol) in CH_2Cl_2 (10 mL) as described for **70**. The residue was purified by column chromatography (4:1 hexanes–EtOAc) to give disaccharide **83** (0.24 g, 89%) as a colorless oil. R_f 0.37 (3:1 hexanes–EtOAc); $[\alpha]_D$ –2.6 (*c* 1.8, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 8.20–7.90 (m, 8 H, Ar), 7.88–7.82 (m, 4 H, Ar), 7.58–7.16 (m, 18 H, Ar), 5.86 (ddd, 1 H, *J* = 6.5, 6.5, 5.0 Hz, H-5'), 5.81 (dd, 1 H, *J* = 5.0, 1.0 Hz, H-3), 5.78 (s, 1 H, H-1'), 5.64 (d, 1 H, *J* = 1.4 Hz, H-2'), 5.54 (dd, 1 H, *J* = 4.3, 1.2 Hz, H-3'), 5.49 (d, 1 H, *J* = 1.0 Hz, H-2), 5.21 (s, 1 H, H-1) 4.93 (dd, 1 H, *J* = 5.0, 4.3 Hz, H-4'), 4.78–4.74 (m, 3 H, H-6_a, H-5, H-6_b), 4.56–4.39 (m, 3 H, H-6_a', H-6_b', H-4), 3.70 (ddd, 1 H, *J* = 9.5, 6.0, 6.0 Hz, octyl OCH_2), 3.48 (ddd, 1 H, *J* = 9.5, 6.0, 6.0 Hz, octyl OCH_2), 1.63–2.05 (s, 3 H, C(=O)CH_3), 1.63–1.59 (m, 2 H, octyl CH_2), 1.28–1.21 (m, 10 H, octyl CH_2), 0.89 (t, 3 H, *J* = 7.0 Hz, octyl CH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 170.4 (C=O), 166.1 (C=O), 165.6 (C=O), 165.6 (C=O), 165.5 (C=O), 165.4 (C=O), 165.2 (C=O), 133.4 (Ar), 133.3 (Ar), 133.2 (Ar), 133.1 (Ar), 133.0 (Ar), 129.92 (Ar), 129.90 (Ar), 129.8 (Ar), 129.7 (Ar), 129.6 (Ar), 129.5 (Ar), 129.1 (Ar), 129.0 (Ar), 128.9 (Ar), 128.8 (Ar), 128.71 (Ar), 128.69 (Ar), 128.67 (Ar), 128.63 (Ar), 128.61 (Ar), 128.5 (Ar), 128.4 (Ar), 128.3

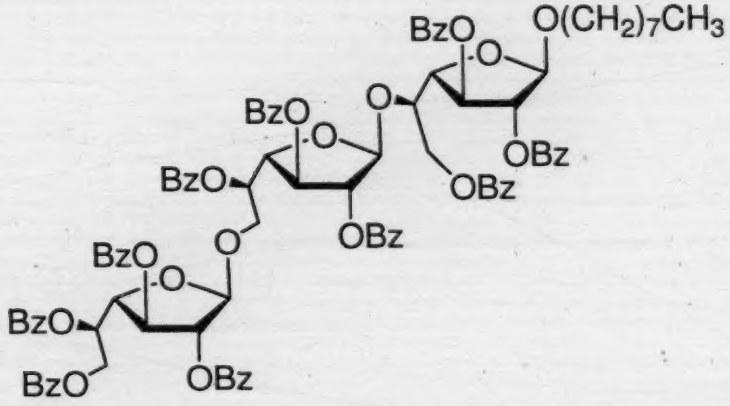
(Ar), 128.2 (Ar), 105.5 (C-1'), 105.3 (C-1), 82.3 (C-2'), 82.1 (C-2), 81.8 (C-4'), 81.7 (C-4), 77.7 (C-3), 77.1 (C-3'), 73.1 (C-5'), 70.3 (C-5), 67.6 (octyl OCH_2), 64.6 (C-6'), 62.9 (C-6), 31.9 (octyl CH_2), 29.6 (octyl CH_2), 29.4 (octyl CH_2), 29.3 (octyl CH_2), 26.2 (octyl CH_2), 22.7 (octyl CH_2), 20.6 ($C(=O)CH_3$), 14.1 (octyl CH_3). ESI-MS *m/z* calcd. for (M + Na) $C_{64}H_{64}O_{18}$: 1143.3984. Found: 1143.3985.



84

Octyl 2,3,5-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→5)-2,3,6-tri-*O*-benzoyl-β-D-galactofuranoside (84). Compound **83** (0.23 g, 0.21 mmol) was dissolved in CH_2Cl_2 (5 mL) and 3% methanolic HCl (12.0 mL). The reaction mixture was stirred for 8 h. The solution was partially concentrated, diluted with CH_2Cl_2 and was washed with saturated $NaHCO_3$, water, and brine. The organic layer was then dried and the solvent was concentrated. The residue was purified by column chromatography (4:1 hexanes–EtOAc) to give disaccharide **84** as a colorless oil (0.05 g, 20%). R_f 0.25 (3:1 hexanes–EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 8.20–7.90 (m, 10 H, Ar), 7.88–7.82 (m, 2 H, Ar), 7.59–7.26 (m, 16 H, Ar), 7.24–7.18 (m, 2 H, Ar), 5.83 (dd, 1 H, J = 5.0, 1.0 Hz, H-3), 5.76 (s, 1 H, H-1'), 5.73 (d, 1 H, J = 1.8 Hz, H-2'), 5.67 (dd, 1 H, J = 5.0, 1.8 Hz, H-3'), 5.46 (d, 1 H, J = 1.3 Hz, H-2), 5.19 (s, 1 H, H-1), 4.77–4.64 (m, 4 H, H-4', H-6_a, H-$6'_a$, H-$6'_b$), 4.53–4.47 (m, 3 H, H-4, H-5, H-6_b), 4.41–4.64 (m, 1 H, H-5'), 3.74 (dt, 1 H, J = 9.0, 7.0

Hz, octyl OCH_2), 3.48 (dt, 1 H, J = 9.0, 7.0 Hz, octyl OCH_2), 1.63 (m, 2 H, octyl CH_2), 1.29–1.23 (m, 10 H, 5 x octyl CH_2), 0.85 (t, 3 H, J = 9.0 Hz, octyl CH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 166.4 (C=O), 166.1 (C=O), 166.0 (C=O), 165.7 (C=O), 165.5 (C=O), 165.1 (C=O), 133.5 (Ar), 133.4 (Ar), 133.3 (Ar), 133.1 (Ar), 133.0 (Ar), 130.02 (Ar), 130.01 (Ar), 130.0 (Ar), 129.9 (Ar), 129.8 (Ar), 129.7 (Ar), 129.6 (Ar), 129.03 (Ar), 129.01 (Ar), 128.9 (Ar), 128.6 (Ar), 128.55 (Ar), 128.49 (Ar), 128.4 (Ar), 128.3 (Ar), 128.22 (Ar), 128.19 (Ar), 128.16 (Ar), 128.15 (Ar), 105.6 (C-1'), 105.5 (C-1), 83.6 (C-2'), 82.1 (C-2), 81.8 (C-4), 81.7 (C-4'), 77.7 (C-3'), 77.5 (C-3), 73.5 (C-5), 69.5 (C-5'), 67.6 (octyl OCH_2), 66.2 (C-6'), 64.6 (C-6), 31.8 (CH_2), 29.5 (octyl CH_2), 29.44 (octyl CH_2), 29.3 (octyl CH_2), 26.2 (octyl CH_2), 22.7 (octyl CH_2), 14.2 (octyl CH_3). ESI-MS m/z calcd. for (M + Na) $C_{62}H_{62}O_{17}$: 1101.3880. Found: 1101.3879.

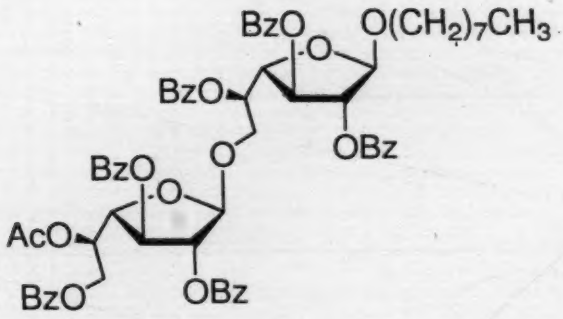


85

Octyl 2,3,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→6)-2,3,5-tri-*O*-benzoyl-β-D-galactofuranosyl)-(1→5)-2,3,6-tri-*O*-benzoyl-β-D-galactofuranoside (85). Method 1: Prepared from **81** (73.0 mg, 0.07 mmol), donor **43** (86.0 mg, 0.12 mmol), NIS (45.6 mg,

0.21 mmol) and AgOTf (3.5 mg, 0.01 mmol) in CH_2Cl_2 (3 mL) as described for **70**. The residue was purified by column chromatography (5:1 hexanes–EtOAc) to give trisaccharide **85** (90.8 mg, 78%) as a colorless oil with the hydrolyzed donor eluting with the product (1:1 **85**–hydrolyzed donor ratio). R_f 0.22 (3:1 hexanes–EtOAc); **One-pot method:** Thioglycoside acceptor **61** (79.2 mg, 0.14 mmol), donor **98** (117.2 mg, 0.16 mmol), and activated, powdered 4 Å molecular sieves (200 mg) in CH_2Cl_2 (10 mL) were cooled to 0 °C. The mixture was stirred for 15 min, and TMSOTf (4.85 μL, 0.03 mmol) was slowly added. Donor consumption was monitored by thin-layer chromatography and after stirring for 1 h, acceptor **40** (16.4 mg, 0.03 mmol) was added to the reaction mixture. This was followed by the addition of NIS (11.1 mg, 0.05 mmol), AgOTf (1.4 mg, 0.005 mmol). The reaction mixture was then stirred for 30 min, neutralized with Et_3N, diluted with CH_2Cl_2 (5 mL) and filtered through Celite. The filtrate was washed successively with a saturated aqueous solution of $Na_2S_2O_3$ (10 mL), saturated aqueous solution of $NaHCO_3$, water, and brine. The organic layer was dried and concentrated. The residue was purified by column chromatography (20:1 toluene–EtOAc) to give trisaccharide **85** (45.4 mg, 42%) as a colorless oil. R_f 0.40 (20:1 toluene–EtOAc); $[\alpha]_D$ –15.2 (*c* 0.2, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H), 8.01–7.92 (m, 16 H, Ar), 7.88–7.76 (m, 4 H, Ar), 7.59–7.17 (m, 24 H, Ar), 7.21–7.17 (m, 6 H, Ar), 6.11 (ddd, 1 H, J = 7.2, 7.2, 3.9 Hz, H-5''), 5.98 (ddd, 1 H, J = 7.6, 7.6, 4.8 Hz, H-5'), 5.82 (dd, 1 H, J = 5.1, 1.6 Hz, H-3"), 5.77 (s, 1 H, H-1'), 5.70 (dd, 1 H, J = 4.8, 1.4 Hz, H-3'), 5.66 (d, 1 H, J = 1.4 Hz, H-2'), 5.63 (dd, 1 H, J = 4.0, 1.5 Hz, H-3), 5.56 (d, 1 H, J = 1.6 Hz, H-2"), 5.33 (d, 1 H, J = 1.5 Hz, H-2), 5.25 (s, 1 H, H-1"), 5.23 (s, 1 H, H-1), 4.93 (dd, 1 H, J = 4.8, 4.8 Hz, H-4'), 4.83–4.69 (m, 7 H, H-4'', H-5, H-6_a, H-6_b, H-6_a'', H-6_a', H-6_b''), 4.53 (dd, 1 H, J

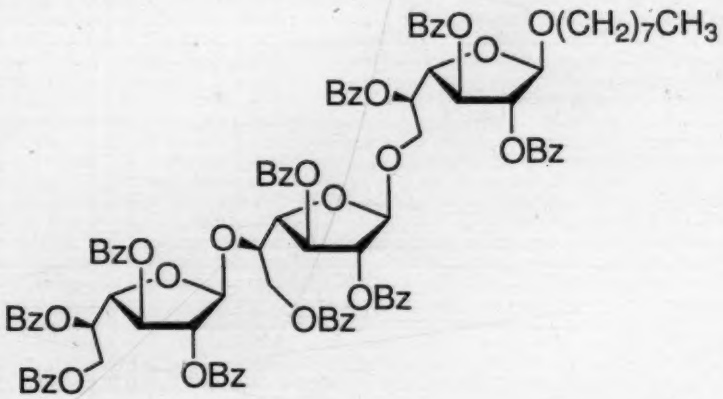
= 5.0, 4.0 Hz, H-4), 4.03 (dd, 1 H, J = 11.5, 7.6 Hz, H-6$_b$'), 3.70 (ddd, 1 H, J = 9.5, 6.7, 6.7 Hz, octyl OCH_2), 3.47 (ddd, 1 H, J = 9.5, 6.7, 6.7 Hz, octyl OCH_2), 1.58–1.53 (m, 2 H, octyl CH_2), 1.36–1.20 (m, 10 H, octyl CH_2), 0.89 (t, 3 H, J = 7.5, octyl CH_3). ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 166.1 (C=O), 166.0 (C=O), 165.9 (C=O), 165.8 (C=O), 165.71 (C=O), 165.70 (C=O), 165.6 (C=O), 165.54 (C=O), 165.52 (C=O), 165.4 (C=O), 133.5 (Ar), 133.4 (Ar), 133.3 (Ar), 133.21 (Ar), 133.19 (Ar), 133.18 (Ar), 133.12 (Ar), 133.06 (Ar), 133.02 (Ar), 133.0 (Ar), 132.8 (Ar), 130.1 (Ar), 130.0 (Ar), 129.94 (Ar), 129.91 (Ar), 129.90 (Ar), 129.85 (Ar), 129.81 (Ar), 129.77 (Ar), 129.75 (Ar), 129.71 (Ar), 129.67 (Ar), 129.58 (Ar), 129.51 (Ar), 129.2 (Ar), 129.1 (Ar), 129.0 (Ar), 128.93 (Ar), 128.91 (Ar), 128.89 (Ar), 128.8 (Ar), 128.52 (Ar), 128.5 (Ar), 128.42 (Ar), 128.40 (Ar), 128.37 (Ar), 128.31 (Ar), 128.25 (Ar), 128.2 (Ar), 106.7 (C-1), 105.5 (C-1"), 105.4 (C-1'), 82.5 (C-4'), 82.4 (C-4"), 82.3 (C-4), 82.2 (C-2'), 82.0 (C-2"), 81.9 (C-2), 77.9 (C-3'), 77.5 (C-3"), 77.4 (C-3), 73.1 (C-5), 71.7 (C-5'), 70.5 (C-5"), 67.7 (C-6'), 67.6 (octyl O*C*H$_2$), 64.6 (C-6''), 63.6 (C-6), 31.9 (octyl *C*H$_2$), 29.6 (octyl *C*H$_2$), 29.4 (octyl *C*H$_2$), 29.3 (octyl *C*H$_2$), 26.2 (octyl *C*H$_2$), 22.7 (octyl *C*H$_2$), 14.1 (octyl *C*H$_3$). ESI-MS *m/z* calcd. for (M + Na) $C_{96}H_{88}O_{26}$: 1679.5413. Found: 1679.5405.



86

Octyl 5-*O*-acetyl-2,3,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→6)-2,3,5-tri-*O*-benzoyl-β-D-galactofuranoside) (86). Prepared from **39** (0.18 g, 0.30 mmol), donor **41** (0.25 g, 0.39 mmol), NIS (0.10 g, 0.45 mmol) and AgOTf (15.4 mg, 0.06 mmol) in CH_2Cl_2 (5 mL) as described for **70**. The residue was purified by column chromatography (4:1 hexanes–EtOAc) to give disaccharide **86** (0.34 g, 99%) as a colorless oil. R_f 0.27 (3:1 hexanes–EtOAc); $[\alpha]_D$ –2.5 (*c* 1.8, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 8.10–7.98 (m, 10 H, Ar), 7.59–7.21 (m, 20 H, Ar), 5.89 (ddd, 1 H, *J* = 7.5, 7.5, 4.0 Hz, H-5'), 5.78 (ddd, 1 H, *J* = 7.5, 7.5, 4.0 Hz, H-5), 5.60 (d, 1 H, *J* = 4.9 Hz, H-3'), 5.47 (dd, 1 H, *J* = 4.2, 1.0 Hz, H-3), 5.44 (d, 1 H, *J* = 1.0 Hz, H-2'), 5.41 (d, 1 H, *J* = 1.0 Hz, H-2), 5.36 (s, 1 H, H-1), 5.27 (s, 1 H, H-1'), 4.69–4.63 (m, 3 H, H-4, H-4', H-6_b), 4.60 (dd, 1 H, *J* = 12.0, 7.5 Hz, H-6_a), 4.19 (dd, 1 H, *J* = 10.5, 7.5 Hz, H-$6'_a$), 4.05 (dd, 1 H, *J* = 10.5, 7.5 Hz, H-$6'_b$), 3.75 (ddd, 1 H, *J* = 9.5, 6.7, 6.7 Hz, octyl O*CH*$_2$), 3.47 (ddd, 1 H, *J* = 9.5, 6.7, 6.7 Hz, octyl O*CH*$_2$), 2.03 (s, 3 H, C(=O)*CH*$_3$), 1.63–1.58 (m, 2 H, octyl *CH*$_2$), 1.28–1.24 (m, 10 H, octyl *CH*$_2$), 0.89 (t, 3 H, *J* = 7.0 Hz, octyl *CH*$_3$); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 170.0 (C=O), 166.0 (C=O), 165.8 (C=O), 165.7 (C=O), 165.6 (C=O), 165.5 (C=O), 165.1 (C=O), 133.5 (Ar), 133.3 (Ar), 133.1 (Ar), 133.0 (Ar), 130.2 (Ar), 130.1 (Ar), 130.0 (Ar), 129.9 (Ar), 129.85 (Ar), 129.80 (Ar), 129.78 (Ar), 129.69 (Ar), 129.66 (Ar),

129.63 (Ar), 129.2 (Ar), 129.1 (Ar), 129.0 (Ar), 128.9 (Ar), 128.6 (Ar), 128.5 (Ar), 128.4 (Ar), 128.39 (Ar), 128.36 (Ar), 128.34 (Ar), 106.2 (C-1), 105.7 (C-1'), 82.2 (C-2'), 81.6 (C-2), 81.5 (C-4), 81.4 (C-4'), 77.5 (C-3'), 77.3 (C-3), 71.5 (C-5'), 69.8 (C-5), 67.8 (octyl OCH_2), 66.3 (C-6), 63.5 (C-6'), 31.9 (octyl CH_2), 29.6 (octyl CH_2), 29.4 (octyl CH_2), 29.3 (octyl CH_2), 26.2 (octyl CH_2), 22.7 (octyl CH_2), 20.8 ($C(=O)CH_3$), 14.1 (octyl CH_3). ESI-MS *m/z* calcd. for (M + Na) $C_{64}H_{64}O_{18}$: 1143.3984. Found: 1143.3983.

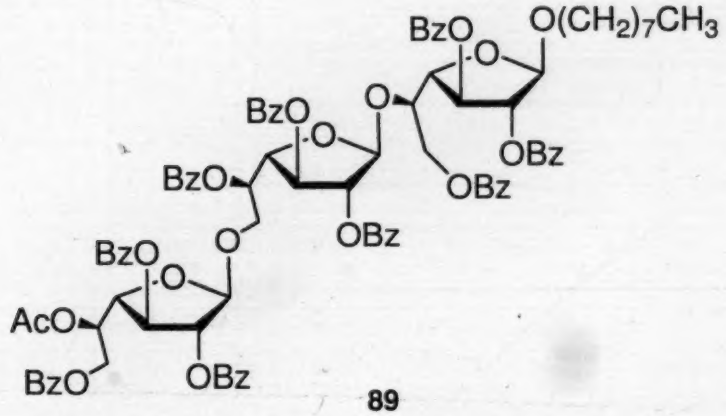


88

Octyl 2,3,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→5)-2,3,6-tri-*O*-benzoyl-β-D-galactofuranosyl)-(1→6)-2,3,5-tri-*O*-benzoyl-β-D-galactofuranoside (88). Method 1: Prepared from **82** (60.7 mg, 0.06 mmol), donor **43** (99.30 mg, 0.14 mmol), NIS (31.6 mg, 0.14 mmol) and AgOTf (2.9 mg, 0.01 mmol) in CH_2Cl_2 (2 mL) as described for **70**. The residue was purified by column chromatography (4:1 hexanes–EtOAc) to give trisaccharide **88** (90.8 mg, 91%) as a clear, colorless oil. R_f 0.22 (3:1 hexanes–EtOAc); **One-pot method:** Thioglycoside acceptor **62** (46.2 mg, 0.08 mmol), donor **98** (74.4 mg, 0.10 mmol), and activated, powdered 4 Å molecular sieves (200.0 mg) in CH_2Cl_2 (7 mL)

were cooled to 0 °C. The suspension was stirred for 15 min before TMSOTf (4.85 μL, 0.03 mmol) was slowly added. Donor consumption was monitored by thin-layer chromatography and after stirring for 1 h, acceptor **39** (29.0 mg, 0.05 mmol) was added to the reaction mixture. This was followed by the addition of NIS (16.2 mg, 0.07 mmol), AgOTf (2.5 mg, 0.01 mmol). The reaction mixture was then stirred for 30 min, neutralized with Et_3N, diluted with CH_2Cl_2 (5 mL) and filtered through Celite. The filtrate was washed successively with a saturated aqueous solution of $Na_2S_2O_3$, saturated aqueous solution of $NaHCO_3$, water, and brine. The organic layer was dried, concentrated and the residue was purified by column chromatography (20:1 toluene–EtOAc) to give trisaccharide **88** (42.0 mg, 41%) as a clear colorless oil. R_f 0.58 (15:1 toluene–EtOAc); $[\alpha]_D$ –15.2 (*c* 0.2, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 8.01–7.82 (m, 16 H, Ar), 7.59–7.17 (m, 34 H, Ar), 6.05 (ddd, 1 H, *J* = 6.5, 6.5, 4.7 Hz, H-5''), 5.91 (ddd, 1 H, *J* = 7.0, 7.0, 3.9 Hz, H-5), 5.83 (dd, 1 H, *J* = 5.1, 1.0 Hz, H-3'), 5.78 (s, 1 H, H-1''), 5.67 (d, 1 H, *J* = 1.0 Hz, H-2''), 5.62 (dd, 1 H, *J* = 4.2, 1.0 Hz, H-3''), 5.60 (d, 1 H, *J* = 5.0 Hz, H-3), 5.46 (d, 1 H, *J* = 1.0 Hz, H-2'), 5.43 (d, 1 H, *J* = 1.0 Hz, H-2), 5.33 (s, 1 H, H-1'), 5.27 (s, 1 H, H-1), 5.05 (dd, 1 H, *J* = 4.7, 4.2 Hz, H-4''), 4.83–4.64 (m, 7 H, H-4', H-5', H-4, H-6_a', H-6_b', H-6_a'', H-6_b''), 4.18 (dd, 1 H, *J* = 10.6, 7.0 Hz, H-6_a), 4.04 (dd, 1 H, *J* = 10.6, 7.0 Hz, H-6_b), 3.72 (ddd, 1 H, *J* = 9.5, 6.7, 6.7 Hz, octyl OCH_2), 3.50 (ddd, 1 H, *J* = 9.5, 6.7, 6.7 Hz, octyl OCH_2), 1.58–1.53 (m, 2 H, octyl CH_2), 1.28–1.20 (m, 10 H, octyl CH_2), 0.89 (t, 3 H, *J* = 7.0 Hz, octyl CH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 166.1 (C=O), 166.04 (C=O), 165.0 (C=O), 165.8 (C=O), 166.7 (C=O), 165.67 (C=O), 165.63 (C=O), 165.60 (C=O), 165.5 (C=O), 165.47 (C=O), 133.5 (Ar), 133.4 (Ar), 133.3 (Ar), 133.28 (Ar), 133.26 (Ar), 133.21 (Ar), 133.2 (Ar), 133.1 (Ar), 133.04 (Ar), 133.01 (Ar), 132.92

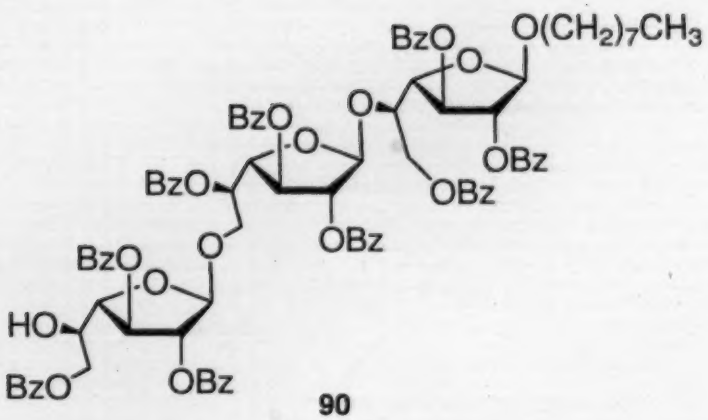
(Ar), 132.86 (Ar), 130.01 (Ar), 130.0 (Ar), 129.93 (Ar), 129.91 (Ar), 129.85 (Ar), 129.83 (Ar), 129.81 (Ar), 129.75 (Ar), 129.73 (Ar), 129.7 (Ar), 129.67 (Ar), 129.6 (Ar), 128.9 (Ar), 128.86 (Ar), 128.80 (Ar), 128.5 (Ar), 128.42 (Ar), 128.40 (Ar), 128.34 (Ar), 128.31 (Ar), 128.2 (Ar), 128.1 (Ar), 106.1 (C-1), 105.7 (C-1"), 105.3 (C-1'), 83.0 (C-4'), 82.5 (C-4"), 82.2 (C-4), 82.1 (C-2'), 82.0 (C-2"), 81.8 (C-2), 77.8 (C-3'), 77.6 (C-3"), 77.5 (C-3), 73.1 (C-5), 71.7 (C-5'), 70.5 (C-5"), 67.8 (C-6), 63.8 (octyl OCH_2), 63.7 (C-6''), 63.6 (C-6'), 31.9 (octyl CH_2), 29.6 (octyl CH_2), 29.4 (octyl CH_2), 29.3 (octyl CH_2), 26.2 (octyl CH_2), 22.7 (octyl CH_2), 14.1 (octyl CH_3). ESI-MS *m/z* calcd. for (M+Na) $C_{96}H_{88}O_{26}$: 1679.5413. Found: 1679.5405.



89

Octyl 5-*O*-acetyl-2,3,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→6)-2,3,5-tri-*O*-benzoyl-β-D-galactofuranosyl)-(1→5)-2,3,6-tri-*O*-benzoyl-β-D-galactofuranoside (89). Prepared from **81** (60.2 mg, 0.06 mmol), donor **42** (46.5 mg, 0.06 mmol), NIS (18.8 mg, 0.08 mmol) and AgOTf (2.8 mg, 0.01 mmol) in CH_2Cl_2 (3 mL) as described for **70**. The residue was purified by column chromatography (5:1 hexanes–EtOAc) to give trisaccharide **89** (69.0 mg, 78%) as a colorless oil. R_f 0.28 (3:1 hexanes–EtOAc); $[\alpha]_D$ –

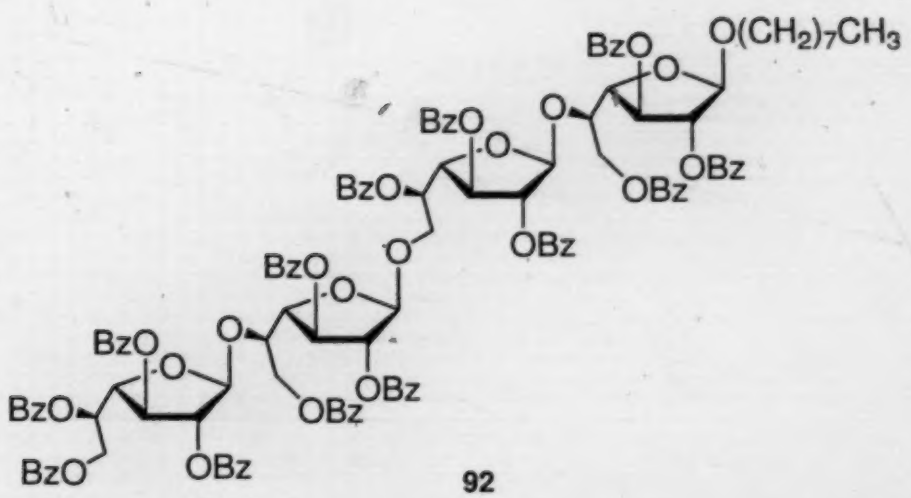
10.8 (*c* 1.5, $CHCl_3$); ^1H NMR (500 MHz,$CDCl_3$, δ_H) 8.20–7.90 (m, 14 H, Ar), 7.88–7.76 (m, 4 H, Ar), 7.59–7.32 (m, 21 H, Ar), 7.22–7.16 (m, 6 H, Ar), 5.95 (ddd, 1 H, *J* = 7.0, 7.0, 4.3 Hz, H-5'), 5.82 (dd, 1 H, *J* = 5.3, 1.6 Hz, H-3"), 5.77 (s, 1 H, H-1'), 5.70 (ddd, 1 H, *J* = 7.9, 7.9, 3.8 Hz, H-5"), 5.64 (dd, 1 H, *J* = 1.0, 0.5 Hz, H-2'), 5.60 (dd, 1 H, *J* = 3.8, 1.0 Hz, H-3'), 5.49 (dd, 1 H, *J* = 1.6, 0.5 Hz, H-2"), 5.42 (dd, 1 H, *J* = 5.1, 1.3 Hz, H-3), 5.33 (d, 1 H, *J* = 1.3 Hz, H-2), 5.21 (br s, 1 H, H-1"), 5.19 (s, 1 H, H-1), 4.91 (dd, 1 H, *J* = 4.3, 3.8 Hz, H-4'), 4.78–4.66 (m, 3 H, H-5, H-6_a, H-6_b), 4.62–4.58 (m, 2 H, H-4, H-6_a'), 4.56–4.49 (m, 2 H, H-4", H-6_b'), 4.11 (dd, 1 H, *J* = 11.0, 7.9 Hz, H-6_a"), 3.98 (dd, 1 H, *J* = 11.0, 7.9 Hz, H-6_b"), 3.70 (ddd, 1 H, *J* = 9.5, 6.7, 6.7 Hz, octyl O*C*H_2), 3.47 (ddd, 1 H, *J* = 9.5, 6.7, 6.7 Hz, octyl O*C*H_2), 1.96 (s, 3 H, C(=O)*C*H_3), 1.58–1.53 (m, 2 H, octyl *C*H_2), 1.34–1.26 (m, 10 H, octyl *C*H_2), 0.89 (t, 3 H, *J* = 7.5 Hz, octyl *C*H_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 170.0 (C=O), 166.1 (C=O), 165.9 (C=O), 165.8 (C=O), 165.6 (C=O), 165.5 (C=O), 165.4 (C=O), 165.4 (C=O), 165.2 (C=O), 164.9 (C=O), 133.44 (Ar), 133.41 (Ar), 133.37 (Ar), 133.33 (Ar), 133.2 (Ar), 133.1 (Ar), 133.0 (Ar), 132.95 (Ar), 132.9 (Ar), 132.8 (Ar), 130.0 (Ar), 129.89 (Ar), 129.88 (Ar), 129.82 (Ar), 129.79 (Ar), 129.77 (Ar), 129.71 (Ar), 129.67 (Ar), 129.66 (Ar), 129.2 (Ar), 129.1 (Ar), 129.0 (Ar), 128.9 (Ar), 128.5 (Ar), 128.5 (Ar), 128.4 (Ar), 128.4 (Ar), 128.3 (Ar), 128.3 (Ar), 128.3 (Ar), 128.2 (Ar), 128.1 (Ar), 106.6 (C-1), 105.5 (C-1"), 105.4 (C-1'), 82.4 (C-4'), 82.3 (C-4"), 82.0 (C-4), 81.8 (C-2'), 81.6 (C-2"), 81.5 (C-2), 77.9 (C-3'), 77.2 (C-3"), 77.2 (C-3), 73.1 (C-5), 71.7 (C-5'), 70.0 (C-5"), 67.7 (C-6"), 67.6 (octyl O*C*H_2), 64.6 (C-6'), 63.6 (C-6), 31.9 (octyl *C*H_2), 29.6 (octyl *C*H_2), 29.4 (octyl *C*H_2), 29.3 (octyl *C*H_2), 26.2 (octyl *C*H_2), 22.7 (octyl *C*H_2), 14.1 (octyl *C*H_3), 20.5 (C(=O)*C*H_3). ESI-MS *m/z* calcd. for (M + Na) $C_{91}H_{86}O_{26}$: 1617.5305. Found: 1617.5260.



90

Octyl 2,3,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→6)-2,3,5-tri-*O*-benzoyl-β-D-galactofuranosyl)-(1→5)-2,3,6-tri-*O*-benzoyl-β-D-galactofuranoside (90). Prepared from **89** (20.0 mg, 0.01 mmol) in CH_2Cl_2 (2 mL) and 3% methanolic HCl (0.73 mL) as described for **81**. The residue was purified by column chromatography (4:1 hexanes–EtOAc) to give disaccharide **90** (18.0 mg, 92%) as a colorless oil. R_f 0.09 (4:1 hexanes–EtOAc); $[\alpha]_D$ –2.8 (*c* 1.5, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 8.20–7.90 (m, 16 H, Ar), 7.88–7.76 (m, 4 H, Ar), 7.59–7.17 (m, 25 H, Ar), 5.95 (ddd, 1 H, *J* = 7.0, 7.0, 4.8 Hz, H-5'), 5.80 (dd, 1 H, *J* = 4.2, 1.0 Hz, H-3"), 5.77 (br s, 1 H, H-1'), 5.63 (dd, 1 H, *J* = 1.5, 0.9 Hz, H-2'), 5.57 (dd, 1 H, *J* = 3.8, 1.5 Hz, H-3'), 5.53 (dd, 1 H, *J* = 4.7, 1.2 Hz, H-3), 5.48 (d, 1 H, *J* = 1.0 Hz, H-2"), 5.37 (d, 1 H, *J* = 1.2 Hz, H-2), 5.22 (s, 1 H, H-1"), 5.16 (s, 1 H, H-1), 4.89 (dd, 1 H, *J* = 4.8, 3.8 Hz, H-4'), 4.76–4.74 (m, 1 H, H-6_a"), 4.73–4.68 (m, 2 H, H-5", H-6_b"), 4.53–4.49 (m, 2 H, H-4, H-6_a), 4.44 (dd, 1 H, *J* = 4.8, 4.2 Hz, H-4"), 4.41 (dd, 1 H, *J* = 11.4, 7.2 Hz, H-6_b), 4.35 (ddd, 1 H, *J* = 7.2, 7.2, 3.4 Hz, H-5), 4.08 (dd, 1 H, *J* = 11.4, 7.0 Hz, H-6_a'), 3.98 (dd, 1 H, *J* = 11.4, 7.0 Hz, H-6_b'), 3.69 (ddd, 1 H, *J* = 9.5, 6.3, 6.3 Hz, octyl OCH_2), 3.47 (ddd, 1 H, *J* = 9.5, 6.3, 6.3 Hz, octyl OCH_2), 1.63–1.60 (m, 2 H, octyl CH_2), 1.28–1.20 (m, 10 H, octyl CH_2), 0.89 (t, 3 H, *J* = 7.0 Hz,

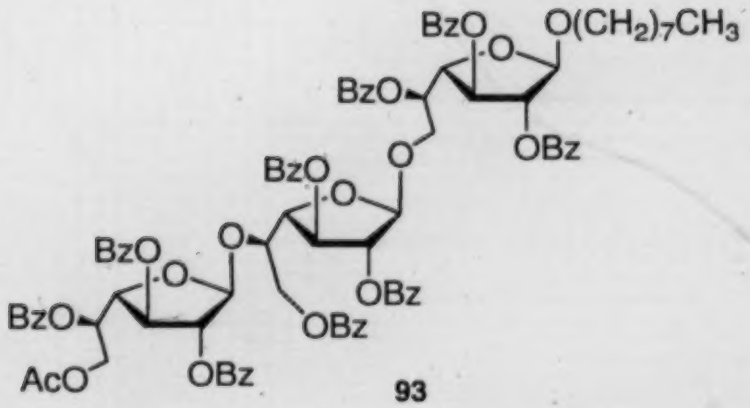
octyl CH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 166.4 (C=O), 166.1 (C=O), 166.0 (C=O), 165.8 (C=O), 165.7 (C=O), 165.5 (C=O), 165.4 (C=O), 165.2 (C=O), 164.9 (C=O), 133.42 (Ar), 133.4 (Ar), 133.33 (Ar), 133.1 (Ar), 133.2 (Ar), 133.12 (Ar), 133.0 (Ar), 132.9 (Ar), 132.86 (Ar), 129.93 (Ar), 129.9 (Ar), 129.86 (Ar), 129.80 (Ar), 129.78 (Ar), 129.76 (Ar), 129.75 (Ar), 129.69 (Ar), 129.66 (Ar), 129.1 (Ar), 129.03 (Ar), 129.0 (Ar), 128.9 (Ar), 128.89 (Ar), 128.51 (Ar), 128.46 (Ar), 128.43 (Ar), 128.36 (Ar), 128.28 (Ar), 128.26 (Ar), 128.15 (Ar), 106.8 (C-1'), 105.5 (C-1"), 105.4 (C-1), 83.7 (C-4'), 82.4 (C-4), 82.3 (C-4"), 82.0 (C-2'), 81.8 (C-2"), 81.4 (C-2), 77.8 (C-3"), 77.3 (C-3'), 77.1 (C-3), 73.3 (C-5), 71.7 (C-5'), 69.3 (C-5''), 67.7 (C-6), 67.6 (C-6), 66.2 (C-6''), 64.7 (octyl OCH_2), 31.9 (octyl CH_2), 29.6 (octyl CH_2), 29.4 (octyl CH_2), 29.3 (octyl CH_2), 26.2 (octyl CH_2), 22.7 (octyl CH_2), 14.1 (octyl CH_3). ESI-MS *m/z* calcd. for (M + Na) $C_{89}H_{84}O_{25}$: 1575.5159. Found: 1575.5157.



92

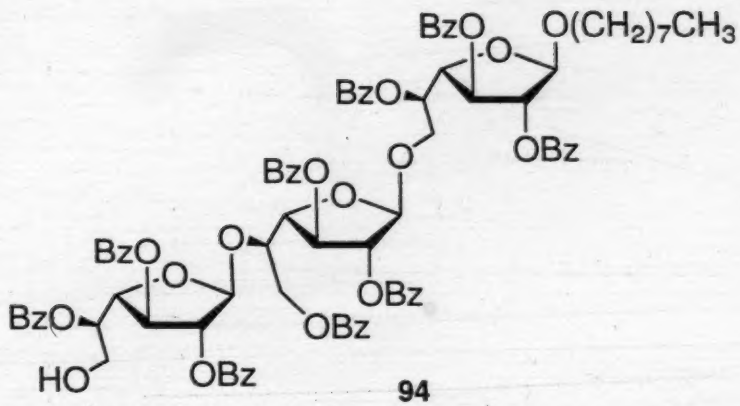
Octyl 2,3,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→5)-2,3,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→6)-2,3,5-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→5)-2,3,6-tri-*O*-benzoyl-β-D-galactofuranoside (92). Method 1: Prepared from **90** (20.0 mg, 0.01 mmol), donor **43** (18.1 mg, 0.03 mmol), NIS (4.3 mg, 0.02 mmol) and AgOTf (6.7 mg, 0.003 mmol) in CH_2Cl_2 (3 mL) as described for **70**. The residue was purified by column chromatography (2:1 hexanes–EtOAc) to give tetrasaccharide **92** (14.9 mg, 70%) as an oil. R_f 0.33 (15:1 toluene–EtOAc). **Method 2:** Prepared from **40** (5.6 mg, 0.01 mmol), donor **110** (23.0 mg, 0.01 mmol), activated, powdered Å molecular sieves (50.0 mg), NIS (6.3 mg, 0.003 mmol) and AgOTf (1.2 mg, 0.0005 mmol) in CH_2Cl_2 (7 mL) as described for **70**. The residue was purified by column chromatography (20:1 toluene–EtOAc) to give tetrasaccharide **92** (13.8 mg, 70%) as an oil. R_f 0.33 (15:1 toluene–EtOAc); $[\alpha]_D$ –7.8 (*c* 0.1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 8.00–7.96 (m, 13 H, Ar), 7.90–7.79 (m, 15 H, Ar), 7.76–7.10 (m, 37 H, Ar), 5.99 (ddd, 1 H, *J* = 7.5, 7.5, 5.3 Hz, H-5'''), 5.93 (ddd, 1 H, *J* = 8.0, 8.0, 4.5 Hz, H-5'), 5.81 (dd, 1 H, *J* = 5.0, 1.5 Hz, H-3''), 5.77–5.76

(m, 3 H, H-3, H-1', H-1'''), 5.65 (br s, 1 H, H-2'), 5.645 (br s, 1 H, H-2'''), 5.61 (dd, 1 H, J = 4.5, 1.3 Hz, H-3'), 5.59 (dd, 1 H, J = 4.5, 1.3 Hz, H-3'''), 5.48 (d, 1 H, J = 1.5 Hz, H-2''), 5.34 (d, 1 H, J = 1.5 Hz, H-2), 5.20 (s, 1 H, H-1''), 5.18 (s, 1 H, H-1), 4.99 (dd, 1 H, J = 5.3, 4.5 Hz, H-4'''), 4.90 (dd, 1 H, J = 4.5, 4.5 Hz, H-4'), 4.76–4.61 (m, 8 H, H-5'', H-5, H-6_a, H-6_b, H-6_a'', H-6_b'', H-6_a''', H-6_b'''), 4.58 (dd, 1 H, J = 6.1, 3.0 Hz, H-4), 4.50 (dd, 1 H, J = 5.3, 3.9 Hz, H-4''), 4.09 (dd, 1 H, J = 11.4, 5.9 Hz, H-6_a'), 3.97 (dd, 1 H, J = 11.4, 5.9 Hz, H-6_b'), 3.67 (ddd, 1 H, J = 9.0, 6.5, 6.5 Hz, octyl OCH_2), 3.46 (ddd, 1 H, J = 9.0, 6.5, 6.5 Hz, octyl OCH_2), 1.56–1.55 (m, 2 H, octyl CH_2), 1.33–1.21 (m, 10 H, octyl CH_2), 0.85 (t, 3 H, J = 6.9 Hz, octyl CH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 166.1 (C=O), 165.92 (C=O), 165.91 (C=O), 165.8 (C=O), 165.7 (C=O), 165.64 (C=O), 165.62 (C=O), 165.5 (C=O), 165.4 (2 x C=O), 165.2 (C=O), 165.1 (C=O), 165.07 (C=O), 133.4 (Ar), 133.3 (Ar), 133.25 (Ar), 133.2 (Ar), 133.1 (Ar), 133.05 (Ar), 133.0 (Ar), 132.94 (Ar), 132.9 (Ar), 132.8 (Ar), 129.93 (Ar), 129.9 (Ar), 129.81 (Ar), 129.79 (Ar), 129.76 (Ar), 129.69 (Ar), 129.66 (Ar), 129.6 (Ar), 129.2 (Ar), 129.0 (Ar), 128.91 (Ar), 128.89 (Ar), 128.72 (Ar), 128.5 (Ar), 128.4 (Ar), 128.36 (Ar), 128.3 (Ar), 128.25 (Ar), 128.21 (Ar), 128.17 (Ar), 128.1 (Ar), 128.08 (Ar), 106.6 (C-1''), 105.49 (C-1'''), 105.46 (C-1'), 105.45 (C-1), 82.9 (C-4), 82.4 (C-4'), 82.3 (C-4''), 82.1 (C-4'''), 82.0 (C-2'''), 82.0 (C-2'), 82.0 (C-2''), 82.0 (C-2), 77.83 (C-3', C-3'''), 77.80 (C-3, C-3''), 73.2 (C-5''), 72.7 (C-5), 71.8 (C-5'''), 70.6 (C-5'), 67.6 (C-6'), 67.5 (octyl OCH_2), 65.2 (C-6'''), 64.7 (C-6), 63.8 (C-6''), 31.8 (octyl CH_2), 29.5 (octyl CH_2), 29.4 (octyl CH_2), 29.2 (octyl CH_2), 26.1 (octyl CH_2), 22.6 (octyl CH_2), 14.1 (octyl CH_3). ESI-MS *m/z* calcd. for (M + Na) $C_{123}H_{110}O_{34}$: 2153.6776. Found: 2154.678.



93

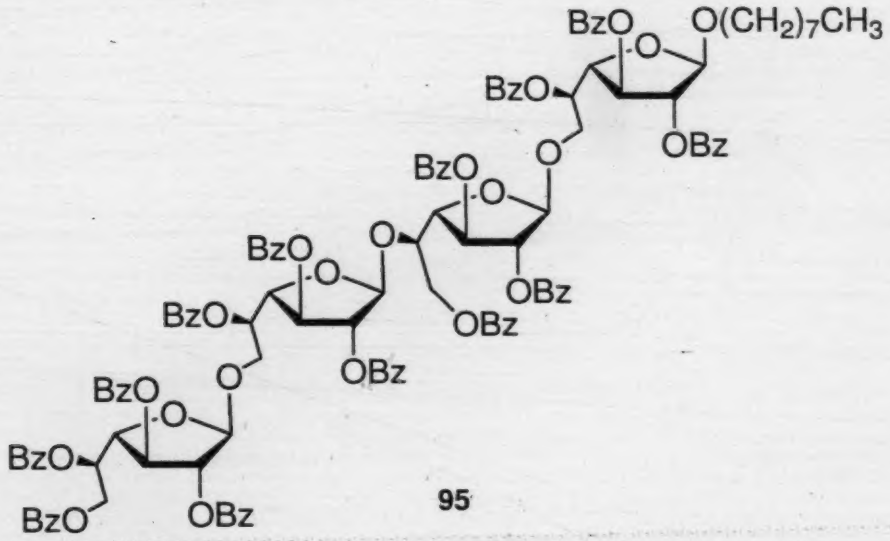
Octyl 6-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→5)-2,3,6-tri-*O*-benzoyl-β-D-galactofuranosyl)-(1→6)-2,3,5-tri-*O*-benzoyl-β-D-galactofuranoside (93). Prepared from **82** (111.4 mg, 0.10 mmol), donor **42** (46.5 mg, 0.06 mmol), NIS (64.9 mg, 0.29 mmol) and AgOTf (5.3 mg, 0.02 mmol) in CH_2Cl_2 (3 mL) as described for **70**. The residue was purified by column chromatography (4:1 hexanes–EtOAc) to give a mixture of trisaccharide **93** and the hydrolyzed donor (1:1 **93: hydrolyzed donor**) as oil. R_f 0.13 (3:1 hexanes–EtOAc). ESI-MS *m/z* calcd. for (M + Na) $C_{91}H_{86}O_{26}$: 1617.5305. Found: 1617.5253.



94

Octyl 2,3,5-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→5)-2,3,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→6)-2,3,6-tri-*O*-benzoyl-β-D-galactofuranoside (94). Prepared from acetylated trisaccharide **93** (not isolated pure) (68.5 mg, 0.04 mmol) in 1.0 mL CH_2Cl_2 (1.0 mL) and 3% methanolic HCl (2.49 mL) as described for **81**. The residue was purified by column chromatography (4:1 hexanes–EtOAc) to give **94** (32.3 mg, 42% over two steps) as a colorless oil. R_f 0.17 (3:1 hexanes–EtOAc); $[\alpha]_D$ –20.4 (*c* 0.8, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$, δ_H) 8.20–7.90 (m, 12 H, Ar), 7.88–7.76 (m, 8 H, Ar), 7.59–7.22 (m, 25 H, Ar), 5.95 (ddd, 1 H, *J* = 7.9, 5.0, 3.8 Hz H-5), 5.80 (dd, 1 H, *J* = 5.3, 1.0 Hz, H-3'), 5.76 (s, 1 H, H-1'), 5.65 (d, 1 H, *J* = 1.0 Hz, H-2'), 5.58 (dd, 1 H, *J* = 6.1, 1.0 Hz, H-3), 5.53 (d, 1 H, *J* = 4.8 Hz, H-3"), 5.52 (ddd, 1 H, *J* = 7.6, 7.6, 3.8 Hz, H-5"), 5.43 (br s, 2 H, H-2'', H-2), 5.34 (s, 1 H, H-1), 5.26 (s, 1 H, H-1''), 4.99 (dd, 1 H, *J* = 4.8, 3.8 Hz, H-4"), 4.77–4.65 (m, 5 H, H-4', H-5', H-6_b', H-6_a', H-4), 4.18 (dd, 1 H, *J* = 11.4, 7.9 Hz, H-6_a), 4.05 (dd, 1 H, *J* = 11.4, 7.9 Hz, H-6_b), 4.01 (dd, 1 H, *J* = 11.4, 5.0 Hz, H-6_a"), (ddd, 1 H, *J* = 11.4, 5.0 Hz, H-6_b"), 3.69 (ddd, 1 H, *J* = 9.6, 6.3, 6.3 Hz, octyl O*C*H_2), 3.47 (ddd, 1 H, *J* = 9.6, 6.3, 6.3 Hz, octyl O*C*H_2), 1.63–1.60 (m, 2 H, octyl CH_2), 1.28–

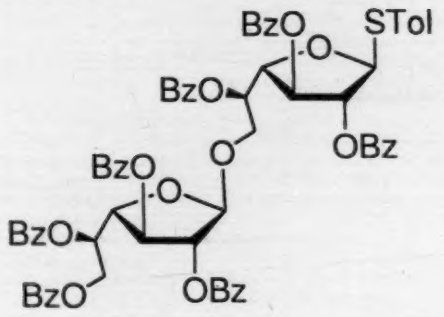
1.21 (m, 10 H, octyl H_2), 0.89 (t, 3 H, J = 7.0, octyl CH_3); ESI-MS m/z calcd. for (M + Na) $C_{89}H_{84}O_{25}$: 1575.5160. Found: 1575.5158.



95

Octyl 2,3,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→6)-2,3,5-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→5)-2,3,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→6)-2,3,5-tri-*O*-benzoyl-β-D-galactofuranoside (95). Prepared from **99** (11.4 mg, 0.02 mmol), donor **39** (46.8 mg, 0.03 mmol), activated, powdered 4 Å molecular sieves (150.0 mg), NIS (12.8 mg, 0.06 mmol) and AgOTf (2.4 mg, 0.01 mmol) in CH_2Cl_2 (15 mL) as described for **70**. The residue was purified by column chromatography (2:1 hexanes–EtOAc) to give tetrasaccharide **95** (30.0 mg, 75%) as a colorless oil. R_f 0.35 (15:1 toluene–EtOAc); $[\alpha]_D$ –10.8 (*c* 0.1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 8.05–7.74 (m, 20 H, Ar), 7.52–7.15 (m, 45 H, Ar), 6.01 (ddd, 1 H, J = 7.5, 7.5, 4.8 Hz, H-5'''), 5.93–5.90 (m, 2 H, H-5'', H-5'), 5.81 (dd, 1 H, J = 4.8, 1.2 Hz, H-3'), 5.77 (s, 1 H, H-1''), 5.63 (dd, 1 H, J = 1.2, 0.7 Hz, H-2''), 5.62 (dd, 1 H, J = 4.8, 1.2 Hz, H-3''), 5.58 (d, 1 H, J = 4.8 Hz, H-3),

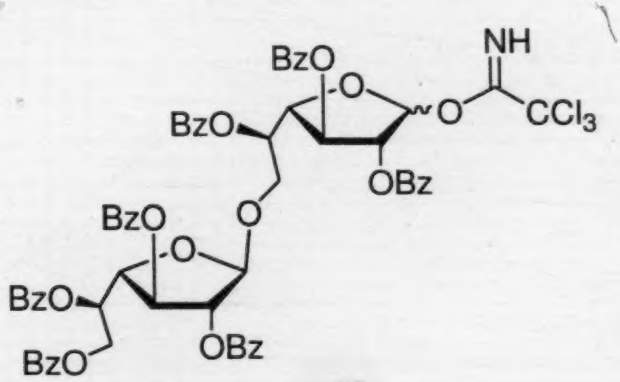
5.54 (d, 1 H, J = 4.8 Hz, H-3'''), 5.44 (d, 1 H, J = 1.2 Hz, H-2'), 5.43 (d, 1 H, J = 1.2 Hz, H-2), 5.323 (d, 1 H, J = 1.2 Hz, H-1'''), 5.318 (d, 1 H, J = 1.2 Hz, H-2'''), 5.26 (s, 1 H, H-1), 5.24 (s, 1 H, H-1'), 4.85 (dd, 1 H, J = 4.8, 4.8 Hz, H-4''), 4.73–4.67 (m, 6 H, H-4''', H-5, H-6_a, H-6_b, H-6_a''', H-6_b'''), 4.66 (dd, 1 H, J = 3.6, 4.8 Hz, H-4), 4.61 (dd, 1 H, J = 5.4, 3.0 Hz, H-4'), 4.16 (dd, 1 H, J = 10.5, 5.2 Hz, H-6'$_a$), 4.12 (dd, 1 H, J = 11.0, 6.0 Hz, H-6_a''), 4.02 (dd, 1 H, J = 10.5, 5.2 Hz, H-6'$_b$), 3.97 (dd, 1 H, J = 11.0, 6.0 Hz, H-6_b''), 3.72 (ddd, 1 H, J = 9.6, 6.6, 6.6 Hz, octyl OCH_2), 3.50 (ddd, 1 H, J = 9.6, 6.6, 6.6 Hz, octyl OCH_2), 1.60–1.55 (m, 2 H, octyl CH_2), 1.33–1.19 (m, 10 H, octyl CH_2), 0.89 (t, 3 H, J = 7.2 Hz, octyl CH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 166.03 (C=O), 166.0 (C=O), 165.83 (C=O), 165.8 (C=O), 165.7 (C=O), 165.63 (C=O), 165.61 (C=O), 165.57 (C=O), 165.5 (C=O), 165.46 (C=O), 165.2 (C=O), 165.1 (C=O), 165.0 (C=O), 133.3 (Ar), 133.24 (Ar), 133.2 (Ar), 133.1 (Ar), 133.0 (Ar), 132.96 (Ar), 132.87 (Ar), 132.85 (Ar), 132.8 (Ar), 129.93 (Ar), 129.89 (Ar), 129.8 (Ar), 129.76 (Ar), 129.7 (Ar), 129.65 (Ar), 129.6 (Ar), 129.2 (Ar), 129.1 (Ar), 129.0 (Ar), 128.9 (Ar), 128.6 (Ar), 128.5 (Ar), 128.47 (Ar), 128.42 (Ar), 128.4 (Ar), 128.35 (Ar), 128.32 (Ar), 128.3 (Ar), 128.25 (Ar), 128.22 (Ar), 128.17 (Ar), 128.12 (Ar), 106.7 (C-1), 106.1 (C-1'''), 105.8 (C-1''), 105.7 (C-1'), 82.7 (C-4), 82.5 (C-4''), 82.1 (C-2), 81.9 (C-2'), 81.84 (C-4'''), 81.83 (C-4'), 81.8 (C-2'''), 81.4 (C-2''), 77.7 (C-3''), 77.5 (C-3), 77.4 (C-3'''), 77.2 (C-3'), 73.2 (C-5), 71.9 (C-5'), 71.3 (C-5''), 70.5 (C-5'''), 67.74 (C-6''), 67.7 (octyl OCH_2), 66.1 (C-6), 65.1 (C-6'), 63.7 (C-6'''), 31.8 (octyl CH_2), 29.45 (octyl CH_2), 29.4 (octyl CH_2), 29.2 (octyl CH_2), 26.1 (octyl CH_2), 22.6 (octyl CH_2), 14.1 (octyl CH_3). ESI-MS m/z calcd. for (M + Na) $C_{123}H_{110}O_{34}$: 2154.678. Found: 2154.68.



97

***p*-Tolyl 2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl-(1→6)-2,3,5-tri-*O*-benzoyl-1-thio-β-D-galactofuranoside (97).** Compound **61** (0.29 g, 0.48 mmol), donor **98** (0.46 g, 0.62 mmol), and activated, powdered 4 Å molecular sieves (0.57 g) in CH_2Cl_2 (25 mL) were cooled at 0 °C. The suspension was stirred for 15 min before TMSOTf (25.8 μL, 0.14 mmol) was added dropwise. The reaction mixture was then stirred for 1 h, neutralized with Et_3N, diluted with CH_2Cl_2 and filtered through Celite. The filtrate was evaporated and the residue was purified by column chromatography (20:1 toluene–EtOAc) to give disaccharide **96** (0.51 g, 90%) as a white semi-solid. R_f 0.37 (20:1 toluene–EtOAc); $[\alpha]_D$ –47.9 (*c* 0.7, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 8.06–8.03 (m, 8 H, Ar), 7.95 (dd, 2 H, *J* = 6.6, 4.0 Hz, Ar), 7.90 (dd, 2 H, *J* = 8.4, 1.2 Hz, Ar), 7.78 (dd, 2 H, *J* = 8.4, 1.2 Hz, Ar), 7.55–7.26 (m, 23 H, Ar), 7.04 (d, 2 H, *J* = 8.0 Hz, Ar), 6.05 (ddd, 1 H, *J* = 7.0, 7.0, 3.7 Hz, H-5'), 5.93 (ddd, 1 H, *J* = 6.3, 6.3, 4.5 Hz, H-5), 5.77 (d, 1 H, *J* = 1.0 Hz, H-1), 5.67 (dd, 1 H, *J* = 4.5, 2.4 Hz, H-3), 5.63 (dd, 1 H, *J* = 2.4, 1.0 Hz, H-2), 5.56 (dd, 1 H, *J* = 5.0, 1.0 Hz, H-3'), 5.42 (d, 1 H, *J* = 1.0 Hz, H-2'), 5.38 (s, 1 H, H-1'), 4.96 (dd, 1 H, *J* = 4.5, 4.5 Hz, H-4), 4.75–4.71 (m, 2 H, H-4', H-6_a'), 4.64 (dd, 1 H, *J* = 11.9, 7.0 Hz, H-6_b'), 4.18 (dd, 1 H, *J* = 10.3, 6.3 Hz, H-6_a), 3.98 (dd, 1 H, *J* = 10.3, 6.3 Hz, H-6_b), 2.24 (s, 3 H, ArCH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 166.1 (C=O),

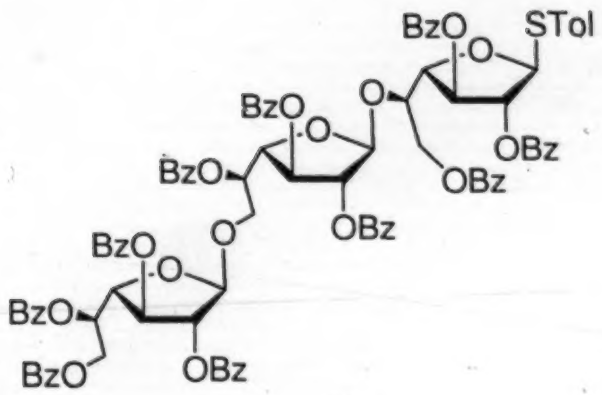
165.73 (C=O), 165.72 (C=O), 165.71 (C=O), 165.5 (C=O), 165.4 (C=O), 165.2 (C=O), 138.0 (Ar), 133.5 (Ar), 133.4 (Ar), 133.34 (Ar), 133.3 (Ar), 133.2 (Ar), 133.1 (Ar), 133.0 (Ar), 132.5 (Ar), 130.0 (Ar), 129.9 (Ar), 129.85 (Ar), 129.83 (Ar), 129.80 (Ar), 129.7 (Ar), 129.6 (Ar), 129.62 (Ar), 129.60 (Ar), 129.5 (Ar), 128.9 (Ar), 128.8 (Ar), 128.50 (Ar), 128.46 (Ar), 128.42 (Ar), 128.40 (Ar), 128.38 (Ar), 128.3 (Ar), 128.32 (Ar), 128.30 (Ar), 106.0 (C-1'), 91.4 (C-1), 82.5 (C-2), 82.02 (C-4'), 82.0 (C-2'), 81.6 (C-4), 77.8 (C-3), 77.6 (C-3'), 71.0 (C-5), 70.3 (C-5'), 65.6 (C-6), 63.8 (C-6') 21.0 (ArCH_3). ESI-MS *m/z* calcd. for (M+Na) $C_{68}H_{56}O_{17}S$: 1199.3130. Found: 1199.3128



98

2,3,5,6-Tetra-*O*-benzoyl-β-D-galactofuranosyl-(1→6)-2,3,5-tri-*O*-benzoyl-D-galactofuranosyl tricholoroacetimidate (98). To a stirred solution of **107** (103.5 mg, 0.10 mmol) and trichloroacetonitrile (48.5 μL, 0.50 mmol) in CH_2Cl_2 (20 mL) cooled to 0 °C, was slowly added DBU (7.21 μL, 0.05 mmol). The reaction mixture was stirred at rt for 2 h and then concentrated at rt. The residue was purified by column chromatography (30:1:0.3 toluene–EtOAc–Et_3N) to furnish imidate **97** (85.0 mg, 85%, α:β = 1:9) as a fluffy solid. R_f 0.60 (10:1 toluene–EtOAc); Data for β-anomer: ^{1}H NMR ($CDCl_3$, 500 MHz, δ_H) 8.76 (s, 1 H, N*H*,), 8.06–7.16 (m, 35 H, Ar), 6.68 (br s, 1 H, H-1),

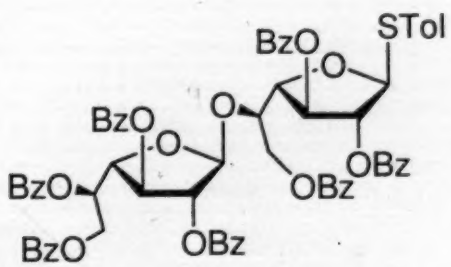
6.05 (ddd, 1 H, J = 6.0, 6.0, 3.7 Hz, H-5'), 5.98 (ddd, 1 H, J = 6.0, 6.0, 4.0 Hz, H-5), 5.73 (dd, 1 H, J = 4.0, 1.8 Hz H-3), 5.72 (d, 1 H, J = 1.8 Hz, H-2), 5.57 (dd, 1 H, J = 5.2, 2.0 Hz, H-3'), 5.36 (s, 1 H, H-1'), 5.35 (d, 1 H, J = 2.0 Hz, H-2'), 4.88 (dd, 1 H, J = 4.0, 4.0 Hz, H-4), 4.77–4.70 (m, 3 H, H-4', H-6a', H-6b'), 4.22 (dd, 1 H, J = 10.0, 6.0 Hz, H-6_a), 4.03 (dd, 1 H, J = 10.0, 6.0 Hz, H-6_b); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 166.0 (C=O), 165.6 (C=O), 165.6 (C=O), 165.44 (C=O), 165.42 (C=O), 165.40 (C=O), 165.0 (C=O), 160.4 (*C*=NH), 133.5 (Ar), 133.4 (Ar), 133.34 (Ar), 133.30 (Ar), 133.2 (Ar), 133.1 (Ar), 133.0 (Ar), 132.5 (Ar), 130.0 (Ar), 129.9 (Ar), 129.82 (Ar), 129.81 (Ar), 129.80 (Ar), 129.7 (Ar), 129.64 (Ar), 129.63 (Ar), 129.60 (Ar), 129.5 (Ar), 128.9 (Ar), 128.8 (Ar), 128.5 (Ar), 128.5 (Ar), 128.42 (Ar), 128.41 (Ar), 128.40 (Ar), 128.34 (Ar), 128.33 (Ar), 128.30 (Ar), 106.7 (C-1'), 103.7 (C-1), 83.4 (C-2), 81.8 (C-2'), 81.6 (C-4'), 80.9 (C-4), 77.5 (C-3), 76.9 (C-3'), 70.9 (C-5), 70.2 (C-5'), 65.6 (C-6'), 63.9 (C-6). ESI-MS *m/z* calcd. for (M + Na) $C_{63}H_{50}Cl_3NO_{18}$: 1236.1991. Found: 1236.2032.



99

***p*-Tolyl 2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl (1→6)-2,3,5-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→5)-2,3,6-tri-*O*-benzoyl-1-thio-β-D-galactofuranoside (99).** Prepared from **62** (0.33 g, 0.27 mmol), donor **97** (0.12 g, 0.21 mmol), and activated, powdered 4 Å molecular sieves (0.35 g), TMSOTf (11.2 μL, 0.06 mmol) in CH_2Cl_2 (25 mL) as described for **96**. The residue was purified by column chromatography (30:1 toluene–EtOAc) to give trisaccharide **99** (0.26 g, 76%) as a white semi-solid. R_f 0.54 (30:1 toluene–EtOAc); $[\alpha]_D$ –40.7 (*c* 2.1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 8.08–7.92 (m, 12 H, Ar), 7.86 (dd, 2 H, *J* = 8.5, 1.5 Hz, Ar), 7.81 (dd, 2 H, *J* = 8.5, 1.5 Hz, Ar), 7.75 (dd, 2 H, *J* = 8.5, 1.5 Hz, Ar), 7.51–7.16 (m, 34 H, Ar), 7.05 (d, 2 H, *J* = 8.5 Hz, Ar), 6.02 (ddd, 1 H, *J* = 7.5, 7.5, 4.0 Hz, H-5''), 5.96–5.91 (m, 3 H, H-5', H-2, H-3''), 5.76 (s, 1 H, H-1), 5.71 (d, 1 H, *J* = 2.5 Hz, H- 1''), 5.68 (dd, 1 H, *J* = 2.5, 2.5 Hz, H-2''), 5.66–5.63 (m, 2 H, H-5, H-3), 5.55 (dd, 1 H, *J* = 5.0, 1.0 Hz, H-3'), 5.34 (d, 1 H, *J* = 1.0 Hz, H-2'), 5.24 (s, 1 H, H-1'), 4.90 (dd, 1 H, *J* = 4.5, 4.5 Hz, H-4), 4.79–4.67 (m, 6 H, H-4', H-4'', H-6_a', H-6_b', H-6_a'', H-6_b''), 4.09 (dd, 1 H, *J* = 11.0, 6.5 Hz, H-6_a), 3.97 (dd, 1 H, *J* = 11.0, 6.5 Hz, H-6_b), 2.37 (s, 3 H, ArCH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 166.1 (C=O), 166.0 (C=O), 165.8 (C=O), 165.7 (C=O), 165.68 (C=O), 165.56 (C=O), 165.52

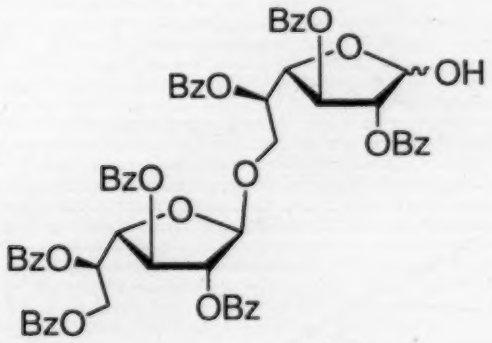
(C=O), 165.3 (C=O), 165.2 (C=O), 165.2 (C=O), 138.0 (Ar), 133.7 (Ar), 133.6 (Ar), 133.52 (Ar), 133.48 (Ar), 133.3 (Ar), 133.29 (Ar), 133.22 (Ar), 133.19 (Ar), 133.12 (Ar), 133.08 (Ar), 133.05 (Ar), 133.02 (Ar), 133.0 (Ar), 132.93 (Ar), 132.89 (Ar), 132.88 (Ar), 132.7 (Ar), 130.1 (Ar), 130.0 (Ar), 129.9 (Ar), 129.82 (Ar), 129.8 (Ar), 129.76 (Ar), 129.72 (Ar), 129.68 (Ar), 129.6 (Ar), 129.5 (Ar), 128.9 (Ar), 128.8 (Ar), 128.6 (Ar), 128.54 (Ar), 128.50 (Ar), 128.32 (Ar), 128.3 (Ar), 128.26 (Ar), 128.24 (Ar), 128.22 (Ar), 106.7 (C-1'), 105.8 (C-1''), 91.1 (C-1), 82.7 (C-2), 82.3 (C-2''), 82.0 (C-4'), 81.9 (C-2'), 81.9 (C-4''), 81.5 (C-4), 77.7 (C-3), 77.4 (C-3'), 77.3 (C-3''), 73.7 (C-5''), 71.8 (C-5), 70.5 (C-5'), 67.5 (C-6'), 64.6 (C-6''), 63.8 (C-6), 21.1 ($ArCH_3$). ESI-MS *m/z* calcd. for (M+Na) $C_{95}H_{78}O_{25}SNa$: 1673.4445. Found: 1673.4441.



106

***p*-Tolyl 2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl-(1→5)-2,3,6-tri-*O*-benzoyl-1-thio-β-D-galactofuranoside (106).** Prepared from **62** (0.26 g, 0.43 mmol), donor **98** (0.40 g, 0.53 mmol), and activated, powdered 4 Å molecular sieves (0.40 g) and TMSOTf (15.43 μL, 0.06 mmol) in CH_2Cl_2 as described for **96**. The residue was purified by column chromatography (30:1 toluene–EtOAc) to give disaccharide **106** (0.43 g, 86%) as a white semi-solid. R_f 0.25 (20:1 toluene–EtOAc); $[\alpha]_D$ –37.8 (*c* 0.7, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$, δ_H) 8.04–7.83 (m, 14 H, Ar), 7.58–7.22 (m, 23 H, Ar), 7.20 (d, 2 H, *J*

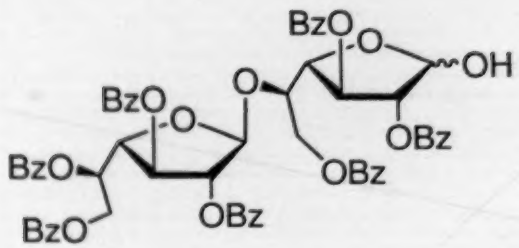
= 8.4 Hz, Ar), 6.01 (ddd, 1 H, *J* = 6.5, 6.5, 4.5 Hz, H-5'), 5.90 (dd, 1 H, *J* = 3.0, 1.8 Hz, H-3), 5.77 (s, 1 H, H-1'), 5.71 (d, 1 H, *J* = 1.8 Hz, H-1), 5.68 (dd, 1 H, *J* = 1.8, 1.8 Hz, H-2), 5.65 (d, 1 H, *J* = 1.2 Hz, H-2'), 5.62 (dd, 1 H, *J* = 4.5, 1.2 Hz, H-3'), 4.99 (dd, 1 H, *J* = 4.5, 4.5 Hz, H-4'), 4.77 (dd, 1 H, *J* = 4.8, 3.0 Hz, H-4), 4.72–4.61 (m, 5 H, H-5, H-6_a, H-6_b, H-6_a', H-6_b'), 2.28 (s, 3 H, ArCH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 166.1 (C=O), 165.9 (C=O), 165.7 (C=O), 165.5 (C=O), 165.5 (C=O), 165.3 (C=O), 165.2 (C=O), 138.1 (Ar), 133.52 (Ar), 133.5 (Ar), 133.3 (Ar), 133.1 (Ar), 133.0 (Ar), 132.9 (Ar), 132.8 (Ar), 130.0 (Ar), 129.9 (Ar), 129.83 (Ar), 129.8 (Ar), 129.72 (Ar), 129.7 (Ar), 129.68 (Ar), 129.66 (Ar), 129.6 (Ar), 129.55 (Ar), 129.4 (Ar), 128.85 (Ar), 128.8 (Ar), 128.76 (Ar), 128.53 (Ar), 128.5 (Ar), 128.4 (Ar), 128.3 (Ar), 128.23 (Ar), 128.21 (Ar), 105.4 (C-1'), 91.2 (C-1), 82.3 (C-4), 82.2 (C-4'), 82.1 (C-2'), 81.5 (C-2), 77.8 (C-3), 77.1 (C-3'), 73.4 (C-5), 70.5 (C-5'), 64.4 (C-6'), 63.8 (C-6), 21.1 (ArCH_3). ESI-MS *m/z* calcd. for (M + Na) $C_{68}H_{56}O_{17}S$: 1199.3130. Found: 1199.3128.



107

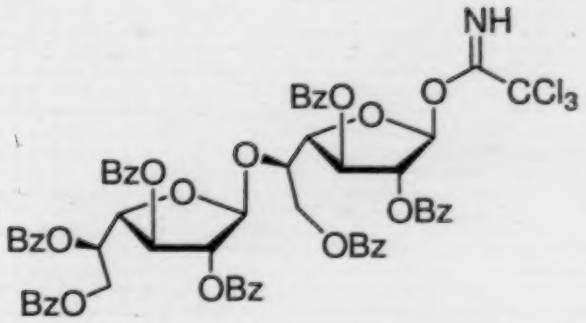
2,3,5,6-Tetra-*O*-benzoyl-β-D-galactofuranosyl-(1→6)-2,3,5-tri-*O*-benzoyl-D-galactofuranose (107). To a solution of thioglycoside **96** (0.22 g, 0.18 mmol) in EtOAc (25 mL), was added NBS (0.35 g, 1.80 mmol) and water (5 mL). The reaction mixture

was allowed to stir for 4 h and then a saturated aqueous solution of sodium $Na_2S_2O_3$ was added. The organic layer was then washed with water, brine, dried and the solvent was evaporated. The crude product was purified by column chromatography (20:1 toluene–EtOAc) to obtain **107** (0.15 g, 74%, α:β = 1:3) as a fluffy, white semi-solid. R_f 0.26 (20:1 toluene–EtOAc); $[\alpha]_D$ +3.0 (*c* 0.9, $CHCl_3$), Data for β-anomer: ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 8.18–7.15 (m, 35 H, Ar), 6.10–5.94 (m, 2 H, H-5, H-5'), 5.81–5.80 (m, 2 H, H-1', H-3), 5.60 (dd, 1 H, *J* = 5.0, 1.5 Hz, H-3'), 5.54 (d, 1 H, *J* = 1.5 Hz, H-2), 5.46 (d, 1 H, *J* = 1.5 Hz, H-2'), 5.34 (s, 1 H, H-l), 5.08–4.65 (m, 4 H, H-4, H-4', H-6_a', H-6_b'), 4.24–3.97 (m, 2 H, H-6_a, H-6_b); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 166.0 (C=O), 165.6 (C=O), 165.6 (C=O), 165.4 (C=O), 165.4 (C=O), 165.4 (C=O), 165.0 (C=O), 133.5 (Ar), 133.4 (Ar), 133.3 (Ar), 133.3 (Ar), 133.2 (Ar), 133.1 (Ar), 133.0 (Ar), 132.5 (Ar), 130.0 (Ar),129.9 (Ar), 129.8 (Ar), 129.8 (Ar), 129.8 (Ar), 129.7 (Ar), 129.64 (Ar), 129.61 (Ar), 129.60 (Ar), 129.5 (Ar), 128.9 (Ar), 128.8 (Ar), 128.52 (Ar), 128.50 (Ar), 128.44 (Ar), 128.43 (Ar), 128.42 (Ar), 128.31 (Ar), 128.30 (Ar), 128.28 (Ar), 104.7 (C-l'), 100.7 (C-l), 83.2 (C-2), 81.8 (C-2'), 81.6 (C-4'), 80.6 (C-4), 77.4 (C-3), 77.1 (C-3'), 70.6 (C-5), 70.3 (C-5'), 64.1 (C-6), 63.3 (C-6'). ESI-MS *m/z* calcd. for (M + Na) $C_{61}H_{50}O_{18}Na$: 1093.2918. Found: 1093.2914.



108

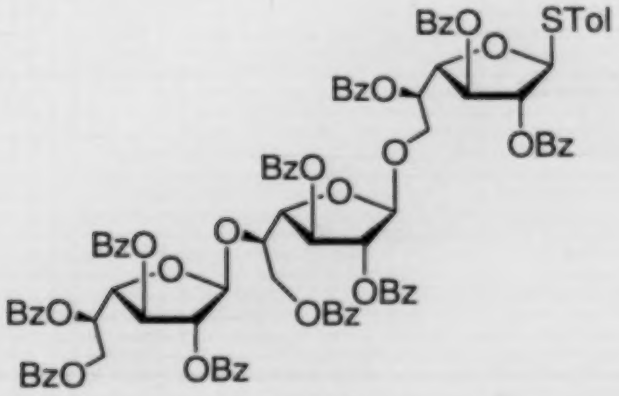
2,3,5,6-Tetra-*O*-benzoyl-β-D-galactofuranosyl-(1→5)-2,3.5-tri-*O*-benzoyl-D-galactofuranose (108). Prepared from thioglycoside **106** (104.2 mg, 0.09 mmol), NBS (157.6 mg, 0.90 mmol), water (2 mL) in EtOAc (25 mL) as described for **107**. The crude product was purified by column chromatography to give **108** (68.0 mg, 72%, α:β = 1:3) as a syrup. R_f 0.11 (15:1 toluene–EtOAc); $[\alpha]_D$ –20.0 (*c* 1.0, $CHCl_3$); Data for β anomer: ^{1}H NMR ($CDCl_3$, 500 MHz, δ_H) 8.20–7.15 (m, 35 H, Ar), 6.05 (ddd, 1 H, *J* = 7.0, 7.0, 4.8 Hz, H-5'), 5.85 (dd, 1 H, *J* = 5.0, 2.0 Hz, H-3'), 5.76 (s, 1 H, H-1'), 5.66–5.63 (m, 3 H, H-1, H-2', H-3), 5.52 (dd, 1 H, *J* = 1.0, 1.5 Hz, H-2), 5.04 (dd, 1 H, *J* = 5.0, 3.5 Hz, H-4), 4.83–4.65 (m, 6 H, H-5, H-6_a, H-6_b, H-6_a', H-6_b', H-4'); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 166.0 (C=O), 165.6 (C=O), 165.6 (C=O), 165.4 (C=O), 165.4 (C=O), 165.4 (C=O), 165.0 (C=O), 133.5 (Ar), 133.4 (Ar), 133.3 (Ar), 133.3 (Ar), 133.2 (Ar), 133.1 (Ar), 133.0 (Ar), 132.5 (Ar), 130.0 (Ar),129.9 (Ar), 129.82 (Ar), 129.80 (Ar), 129.78 (Ar), 129.7 (Ar), 129.6 (Ar), 129.64 (Ar), 129.60 (Ar), 129.5 (Ar), 128.9 (Ar), 128.8 (Ar), 128.5 (Ar), 128.5 (Ar), 128.44 (Ar), 128.43 (Ar), 128.40 (Ar), 128.33 (Ar), 128.31 (Ar), 128.30 (Ar), 106.1 (C-1'), 100.8 (C-1), 82.7 (C-2'), 82.5 (C-2), 82.4 (C-4'), 81.8 (C-4), 77.7 (C-3'), 76.6 (C-3), 76.3 (C-5), 70.5 (C-5'), 64.3 (C-6'), 61.6 (C-6). ESI-MS *m/z* calcd. for (M + Na) $C_{61}H_{50}O_{18}$: 1093.2907. Found: 1093.2904.



109

2,3,5,6-Tetra-*O*-benzoyl-β-D-galactofuranosyl-(1→5)-2,3,6-tri-*O*-benzoyl-D-galactofuranosyl tricholoroacetimidate (109). To a stirred solution of **108** (48.0 mg, 0.04 mmol) and trichloroacetonitrile (33.7 μL, 0.33 mmol) in CH_2Cl_2 (5 mL) cooled to 0 °C, was slowly added DBU (3.41 μL, 0.02mmol). The reaction mixture was stirred at rt for 3 h and then concentrated. The residue was purified by column chromatography (30:1:0.3 toluene–EtOAc–Et_3N) to furnish imidate **109** (40.2 mg, 74%, α:β = 1:9) as a fluffy solid. R_f 0.60 (10:1 toluene–EtOAc); Data for β-anomer: ^{1}H NMR ($CDCl_3$, 500 MHz, δ_H) 8.76 (s, 1 H, N*H*,), 8.06–7.16 (m, 35 H, Ar), 6.68 (br s, 1 H, H-1), 6.01 (ddd, 1 H, *J* = 6.5, 6.5, 5.0 Hz, H-5'), 5.90 (dd, 1 H, *J* = 3.0, 1.8 Hz H-3), 5.77 (s, 1 H, H-1'), 5.72 (dd, 1 H, *J* = 1.8, 1.8 Hz, H-2), 5.65 (d, 1 H, *J* = 1.2 Hz, H-2'), 5.62 (d, 1 H, *J* = 4.5 Hz, H-3'), 5.01 (dd, 1 H, *J* = 5.0, 4.5 Hz, H-4'), 4.87 (d, 1 H, *J* = 4.8, 3.0 Hz, H-4), 4.82–4.78 (m, 5 H, H-5, H-6_a', H-6_b'), 4.21 (dd, 1 H, *J* = 10.6, 6.0 Hz, H-6_a), 4.03 (dd, 1 H, *J* = 10.6, 6.0 Hz, H-6_b); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 166.0 (C=O), 165.6 (C=O), 165.6 (C=O), 165.44 (C=O), 165.42 (C=O), 165.40 (C=O), 165.0 (C=O), 160.4 (*C*=NH), 133.5 (Ar), 133.4 (Ar), 133.34 (Ar), 133.30 (Ar), 133.2 (Ar), 133.1 (Ar), 133.0 (Ar), 132.5 (Ar), 130.0 (Ar), 129.9 (Ar), 129.82 (Ar), 129.81 (Ar), 129.80 (Ar), 129.7 (Ar), 129.64 (Ar), 129.63 (Ar), 129.60 (Ar), 129.5 (Ar), 128.9 (Ar), 128.8 (Ar), 128.5 (Ar), 128.5

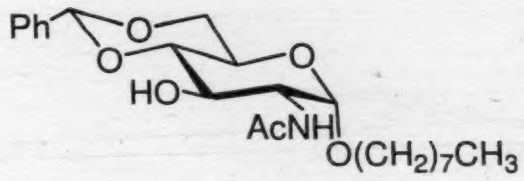
(Ar), 128.42 (Ar), 128.41 (Ar), 128.40 (Ar), 128.32 (Ar), 128.31 (Ar), 128.30 (Ar), 106.7 (C-l'), 105.1 (C-l), 83.4 (C-2), 81.8 (C-2'), 81.6 (C-4'), 80.9 (C-4), 77.5 (C-3), 76.9 (C-3'), 70.9 (C-5), 70.2 (C-5'), 65.6 (C-6'), 63.9 (C-6). ESI-MS *m/z* calcd. for (M + Na) $C_{63}H_{50}Cl_3NO_{18}$: 1236.1991. Found: 1236.2032.



110

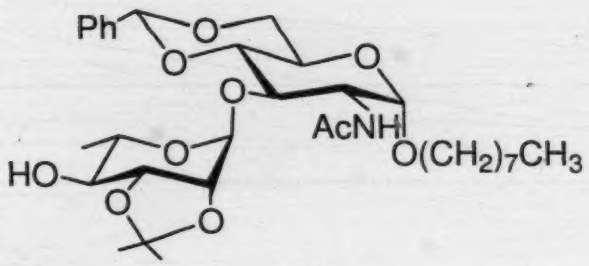
***p*-Tolyl 2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl-(1→5)-2,3,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→6)-2,3,5-tri-*O*-benzoyl-1-thio-β-D-galactofuranoside (110).** Prepared from **61** (13.7 mg, 0.02 mmol), donor **109** (36.2 mg, 0.03 mmol), activated, powdered 4 Å molecular sieves (35.0 mg) and TMSOTf (1.24 μL, 0.006 mmol) in CH_2Cl_2 (5 mL) as described for **96**. The residue was purified by column chromatography (30:1 toluene–EtOAc) to give trisaccharide **110** (27.2 mg, 72%) as a white semi-solid R_f 0.33 (15:1 toluene–EtOAc); $[\alpha]_D$ –8.3 (*c* 0.2, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 8.08–7.92 (m, 9 H, Ar), 7.86 (dd, 2 H, *J* = 8.5, 1.5 Hz, Ar), 7.81 (dd, 2 H, *J* = 8.5, 1.5 Hz, Ar), 7.75 (dd, 2 H, *J* = 8.5, 1.5 Hz, Ar), 7.51–7.16 (m, 37 H, Ar), 7.05 (d, 2 H, *J* = 8.5 Hz, Ar), 6.04 (ddd, 1 H, *J* = 7.5, 7.5, 4.5 Hz, H-5''), 5.91 (ddd, 1 H, *J* = 6.5, 6.5, 4.4 Hz, H-5), 5.80 (br s, 2 H, H-1'', H-3'), 5.73 (d, 1 H, *J* = 1.5 Hz, H-1), 5.69 (d, 1 H, *J* = 1.5

Hz, H-2''), 5.64 (dd, 1 H, J = 4.4, 1.5 Hz, H-3), 5.62 (dd, 1 H, J = 1.5, 1.5 Hz, H-2), 5.61 (dd, 1 H, J = 3.5, 1.5 Hz, H-3''), 5.46 (d, 1 H, J = 1.3 Hz, H-2'), 5.28 (s, 1 H, H-1'), 5.06 (dd, 1 H, J = 4.5, 3.5 Hz, H-4''), 4.91 (dd, 1 H, J = 4.4, 4.4 Hz, H-4), 4.76 (dd, 1 H, J = 12.0, 7.5 Hz, H-6_a''), 4.71–4.64 (m, 4 H, H-5', H-6_b'', H-6_a', H-6_b'), 4.59 (dd, 1 H, J = 4.7, 3.5 Hz, H-4'), 4.14 (dd, 1 H, J = 10.2, 6.5 Hz, H-6_a), 3.93 (dd, 1 H, J = 10.2, 6.5 Hz, H-6_b), 2.20 (s, 3 H, ArCH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 166.0 (C=O), 166.0 (C=O), 165.9 (C=O), 165.69 (C=O), 165.67 (C=O), 165.5 (C=O), 165.5 (C=O), 165.3 (C=O), 165.2 (C=O), 165.1 (C=O), 137.0 (Ar), 133.4 (Ar), 133.35 (Ar), 133.26 (Ar), 133.2 (Ar), 133.14 (Ar), 133.1 (Ar), 133.0 (Ar), 132.9 (Ar), 132.86 (Ar), 132.3 (Ar), 130.1 (Ar), 130.0 (Ar), 129.96 (Ar), 129.93 (Ar), 129.84 (Ar), 129.82 (Ar), 129.82 (Ar), 129.8 (Ar), 129.72 (Ar), 129.68 (Ar), 129.67 (Ar), 129.63 (Ar), 129.6 (Ar), 129.0 (Ar), 128.92 (Ar), 128.9 (Ar), 128.83 (Ar), 128.8 (Ar), 128.5 (Ar), 128.43 (Ar), 128.41 (Ar), 128.34 (Ar), 128.3 (Ar), 128.2 (Ar), 128.1 (Ar), 105.9 (C-1''), 105.2 (C-1'), 91.3 (C-1), 83.2 (C-4'), 82.5 (C-2), 82.1 (C-2''), 81.9 (C-4''), 81.7 (C-4), 81.5 (C-2'), 77.9 (C-3''), 77.8 (C-3), 77.6 (C-3'), 72.6 (C-5'), 70.9 (C-5''), 70.6 (C-5), 65.5 (C-6), 65.0 (C-6'), 63.8 (C-6''), 29.8 (ArCH_3). ESI-MS m/z calcd. for (M + Na) $C_{95}H_{78}O_{25}S$: 1673.4445. Found: 1673.4441.



111

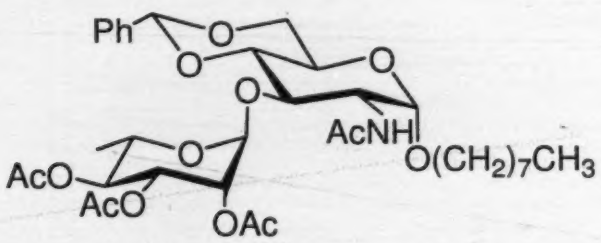
Octyl 2-Acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside (111). To a suspension of **119** (1.64 g, 4.92 mmol) in CH_3CN (35 mL) were added *p*-TsOH (46.8 mg, 0.25 mmol) and α,α'-dimethoxytoluene (3.80 mL, 24.6 mmol). The reaction mixture was then stirred at rt for 3 h, diluted with CH_2Cl_2, neutralized with Et_3N and concentrated. The residue was recrystallized from CH_3CN to give **111** (2.17 g, 73%) as a white solid. R_f 0.61 (10:1 CH_2Cl_2–MeOH); m.p. 187–189 °C; $[\alpha]_D$ +52.7 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.52–7.50 (m, 2 H, Ar), 7.39–7.34 (m, 3 H, Ar), 5.85 (d, 1 H, J = 8.8 Hz, NH), 5.67 (s, 1 H, PhC*H*), 4.81 (d, 1 H, J = 3.8 Hz, H-1), 4.26 (dd, 1 H, J = 9.7, 4.2 Hz, H-6_a), 4.21 (ddd, 1 H, J = 9.0, 8.8, 3.8 Hz, H-2), 3.92 (dd, 1 H, J = 9.0, 9.0 Hz, H-3), 3.84–3.68 (m, 3 H, H-6_b, H-5, octyl OCH_2), 3.58 (dd, 1 H, J = 9.0, 9.0 Hz, H-4), 3.42 (ddd, 1 H, J = 9.8, 6.7, 6.7 Hz, octyl OCH_2), 2.05 (s, 3 H, C(=O)CH_3), 1.62–1.59 (m, 2 H, octyl CH_2), 1.37–1.26 (m, 10 H, octyl CH_2), 0.90 (t, 3 H, J = 7.0 Hz, octyl CH_3); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 171.3 (C=O), 137.1 (Ar), 129.2 (Ar), 128.3 (Ar), 126.3 (Ar), 101.9 (PhCH), 97.7 (C-1), 82.2 (C-4), 70.7 (C-3), 68.9 (C-6), 68.3 (octyl OCH_2), 62.5 (C-5), 54.2 (C-2), 31.8 (octyl CH_2), 29.4 (octyl CH_2), 29.3 (octyl CH_2), 29.2 (octyl CH_2), 26.2 (octyl CH_2), 23.3 (C(=O)CH_3), 22.6 (octyl CH_2), 14.1 (octyl CH_3). ESI-MS *m/z* calcd. for ($M + H^+$) $C_{23}H_{36}NO_6$: 422.2537. Found: 422.2533.



112

Octyl 2,3-Isopropylidene-α-L-rhamnopyranosyl-(1→3)-2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside (112). To a solution of **118** (50.0 mg, 0.09 mmol) in acetone (5 mL) was added 2,2-dimethoxypropane (27.03 μL, 0.22 mmol) and (1*S*)-(+)-camphorsulfonic acid (2.1 mg, 0.009 mmol). After 3 h of stirring, the pH of the solution was adjusted to 7 by adding Et_3N. Concentration gave a crude oil that was subsequently dissolved in CH_2Cl_2 (10 mL), washed with a saturated aqueous solution of $NaHCO_3$ and water. The organic layer was dried, concentrated to an oil and the residue was purified by column chromatography (2:1 hexanes–EtOAc) to furnish **112** (46.0 mg, 86%) as a white solid. R_f 0.59 (10:1 CH_2Cl_2–MeOH); $[\alpha]_D$ +2.6 (*c* 0.5, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.48–7.46 (m, 2 H, Ar), 7.35–7.33 (m, 3 H, Ar), 5.69 (d, 1 H, *J* = 10.0 Hz, NH), 5.51 (s, 1 H, PhC*H*), 5.05 (s, 1 H, H-1'), 4.71 (d, 1 H, *J* = 3.8 Hz, H-1), 4.40 (ddd, 1 H, *J* = 10.0, 10.0, 3.8 Hz, H-2), 4.27 (dd, 1 H, *J* = 10.0, 10.0 Hz, H-3), 4.10 (d, 1 H, *J* = 6.5 Hz, H-2'), 4.01 (dd, 1 H, *J* = 7.3, 6.5 Hz, H-3'), 3.99 (dd, 1 H, *J* = 10.0, 10.0 Hz, H-4), 3.88–3.68 (m, 4 H, H-6_a, H-6_b, H-5', octyl OCH_2), 3.59 (ddd, 1 H, J = 10.0, 10.0, 5.0 Hz, H-5), 3.40 (ddd, 1 H, *J* = 9.8, 6.6, 6.6 Hz, octyl OCH_2), 3.25 (dd, 1 H, *J* = 10.0, 7.3 Hz, H-4'), 2.02 (s, 3 H, C(=O)CH_3), 1.62–1.60 (m, 2 H, octyl OCH_2), 1.47 (s, 3 H, isopropylidene CH_3), 1.32–1.26 (m, 13 H, octyl CH_2, isopropylidene CH_3), 0.90

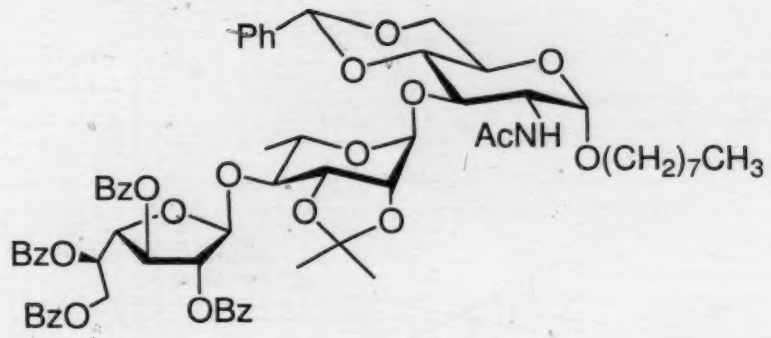
(t, 3 H, J = 6.9 Hz, octyl CH_3), 0.80 (d, 3 H, J = 6.2 Hz, H-6') ; ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 169.8 (C=O), 137.1 (Ar), 129.1 (Ar), 128.1 (Ar), 126.3 (Ar), 109.3 ($(CH_3)_2C$), 102.0 (PhCH), 98.3 (C-1'), 98.2 (C-1), 80.0 (C-4), 78.1 (C-3), 75.9 (C-2'), 75.0 (C-3'), 74.5 (C-4'), 68.9 (C-6), 68.3 (octyl OCH_2), 66.1 (C-5), 63.2 (C-5'), 53.6 (C-2), 31.8 (octyl CH_2), 29.4 (octyl CH_2), 29.3 (octyl CH_2), 29.2 (octyl CH_2), 27.9 (isopropylidene CH_3), 26.2 (octyl CH_2), 26.1 (isopropylidene CH_3), 23.4 (C(=O)CH_3), 22.6 (octyl CH_2), 16.8 (C-6'), 14.1 (octyl CH_3). ESI-MS m/z calcd. for (M + Na) $C_{32}H_{49}NO_{10}$: 630.3249. Found: 630.3249.



114

Octyl 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside (114). Prepared from **111** (0.13 g, 0.31 mmol), donor **113**[127](0.13 g, 0.40 mmol), activated, powdered 4 Å molecular sieves (365.0 mg), NIS (0.10 g, 0.46 mmol) and AgOTf (15.7 mg, 0.06 mmol) in CH_2Cl_2 (10 mL) as described for **70**. The residue was purified by column chromatography (1:1 hexanes–EtOAc) to afford disaccharide **114** (0.19 g, 89%) as a white solid. R_f 0.28 (1:1 hexanes–EtOAc); $[\alpha]_D$ +32.5 (c 0.5, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 7.52–7.50 (m, 2 H, Ar), 7.39–7.34 (m, 3 H, Ar), 5.63 (d, 1 H, J = 10.0 Hz, NH), 5.56 (s, 1 H, PhCH), 5.32 (dd, 1 H, J = 10.0, 3.5 Hz, H-3'), 4.98 (dd, 1 H, J = 3.5, 1.7 Hz, H-2'), 4.95–

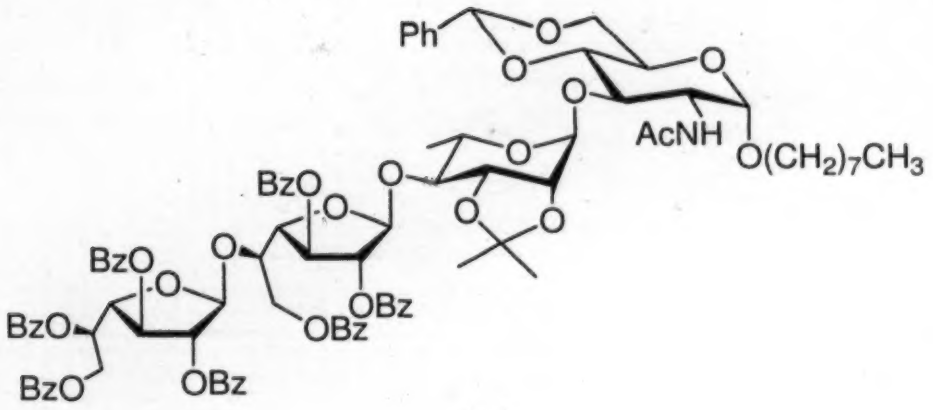
4.91 (m, 2 H, H-1', H-4'), 4.71 (d, 1 H, J = 3.6 Hz, H-1), 4.42 (ddd, 1 H, J = 10.0, 10.0, 3.6 Hz, H-2), 4.26 (ddd, 1 H, J = 10.0, 10.0, 4.5 Hz, H-5), 4.08–4.05 (m, 1 H, H-5'), 3.88 (dd, 1 H, J = 10.0, 10.0 Hz, H-3), 3.83 (dd, 1 H, J = 10.0, 4.5 Hz, H-6_a), 3.76 (dd, 1 H, J = 10.0, 10.0 Hz, H-4), 3.71–3.65 (m, 2 H, H-6_b, octyl OCH_2), 3.38 (ddd, 1 H, J = 9.8, 6.7, 6.7 Hz, octyl OCH_2), 2.09 (s, 3 H, C(=O)CH_3), 2.06 (s, 3 H, C(=O)CH_3), 1.98 (s, 3 H, C(=O)CH_3), 1.96 (s, 3 H, C(=O)CH_3), 1.61–1.58 (m, 2 H, octyl CH_2), 1.32–1.24 (m, 10 H, octyl CH_2), 0.89 (t, 3 H, J = 7.0 Hz, octyl CH_3), 0.69 (d, 3 H, J = 6.5 Hz, H-6'); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 170.3 (C=O), 170.1 (C=O), 170.0 (C=O), 169.8 (C=O), 137.2 (Ar), 129.1 (Ar), 128.1 (Ar), 126.4 (Ar), 102.0 (PhCH), 98.3 (C-1), 97.8 (C-1'), 80.3 (C-4), 76.0 (C-3), 71.5 (C-4'), 70.9 (C-2'), 69.0 (C-6), 68.5 (C-3'), 68.3 (octyl OCH_2), 66.3 (C-5'), 63.1 (C-5), 53.0 (C-2), 31.8 (octyl CH_2), 29.4 (octyl CH_2), 29.3 (octyl CH_2), 29.2 (octyl CH_2), 26.2 (octyl CH_2), 22.6 (octyl CH_2), 20.9 (2 x C(=O)CH_3), 20.7 (C(=O)CH_3), 16.6 (C-6'), 14.1 (octyl CH_3). ESI-MS m/z calcd. for (M + H^+) $C_{35}H_{52}NO_{13}$: 694.3433. Found: 694.3408.



115

Octyl 2,3,4,5-tetra-*O*-benzoyl-β-D-galactofuranosyl-(1→4)-2,3-isopropylidene-α-L-rhamnopyranosyl-(1→3)-2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside (115). Prepared from **112** (44.8 mg, 0.07 mmol), donor **43** (56.9 mg, 0.08 mmol), activated, powdered 4 Å molecular sieves (70.0 mg), NIS (24.9 mg, 0.11 mmol) and AgOTf (3.8 mg, 0.01 mmol) in CH_2Cl_2 (5 mL) as described for **70**. The residue was purified by column chromatography (10:1 toluene–EtOAc) to give trisaccharide **115** (74.0 mg, 85%) as a white solid. R_f 0.18 (10:1 toluene–EtOAc); $[\alpha]_D$ –32.4 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 8.03–7.92 (m, 8 H, Ar), 7.57–7.25 (m, 15 H, Ar), 7.25–7.16 (m, 2 H, Ar), 6.05 (ddd, 1 H, *J* = 7.5, 7.5, 4.8 Hz, H-5''), 5.78 (s, 1 H, H-1''), 5.70 (d, 1 H, *J* = 10.0 Hz, NH), 5.58 (dd, 1 H, *J* = 4.8, 1.0 Hz, H-3''), 5.53 (d, 1 H, *J* = 5.0 Hz, PhC*H*), 5.52 (d, 1 H, *J* = 1.0 Hz, H-2''), 5.10 (s, 1 H, H-1'), 4.73–4.71 (m, 3 H, H-1, H-6_a'', H-6_b''), 4.59 (dd, 1 H, *J* = 4.8, 4.8 Hz, H-4''), 4.42 (ddd, 1 H, *J* = 10.0, 10.0, 3.7 Hz, H-2), 4.31–4.26 (m, 2 H, H-3', H-5), 4.09–4.06 (m, 1 H, H-2'), 4.02–3.93 (m, 2 H, H-3, H-6_a), 3.85 (dd, 1 H, *J* = 9.7, 4.4 Hz, H-6_b), 3.78–3.69 (m, 2 H, H-5', octyl OCH_2), 3.64–3.53 (m, 2 H, H-4, H-4'), 3.41 (ddd, 1 H, *J* = 9.5, 6.7, 6.7 Hz, octyl OCH_2), 3.33 (s, 3 H, C(=O)CH_3), 1.67–1.63 (m, 2 H, octyl CH_2), 1.48 (s, 3 H, isopropylidene CH_3), 1.35–1.25 (m, 13 H, isopropylidene CH_3, octyl CH_2), 0.92 (t, 3 H, *J*

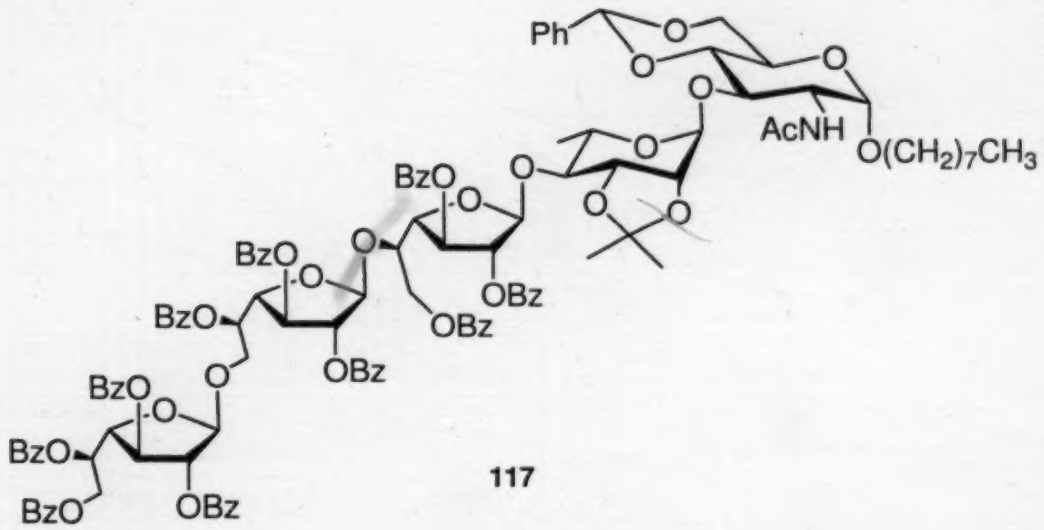
= 6.9 Hz, octyl CH_3), 0.82 (d, 3 H, J = 6.2 Hz, H-6'); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 169.8 (C=O), 166.0 (C=O), 165.7 (2 x C=O), 165.3 (C=O), 137.1 (Ar), 133.4 (Ar), 133.3 (Ar), 133.2 (Ar), 133.0 (Ar), 130.0 (Ar), 129.9 (Ar), 129.8 (Ar), 129.7 (Ar), 129.5 (Ar), 129.4 (Ar), 129.1 (Ar), 129.0 (Ar), 128.9 (Ar), 128.5 (Ar), 128.4 (Ar), 128.3 (Ar), 128.0 (Ar), 126.2 (Ar), 109.4 ($(CH_3)_2C$), 104.1 (C-1''), 101.7 (PhCH), 98.2 (C-1), 98.0 (C-1'), 82.2 (C-2''), 81.5 (C-4''), 80.0 (C-4), 78.1 (C-3''), 77.7 (C-3'), 76.5 (C-2'), 76.2 (C-3), 74.9 (C-4'), 70.4 (C-5''), 68.9 (C-6), 68.3 (octyl OCH_2), 64.4 (C-5), 63.4 (C-6''), 63.2 (C-5'), 53.6 (C-2), 31.8 (octyl CH_2), 29.4 (octyl CH_2), 29.3 (octyl CH_2), 29.24 (octyl CH_2), 29.21 (octyl CH_2), 27.8 ($C(=O)CH_3$), 26.4 (isopropylidene CH_3), 26.2 (isopropylidene CH_3), 22.7 (octyl CH_2), 17.2 (C-6'), 14.1 (octyl CH_3). ESI-MS *m/z* calcd. for (M + Na) $C_{66}H_{75}NO_{19}Na$: 1208.4826. Found: 1208.4831.



116

Octyl 2,3,4,5-Tetra-*O*-benzoyl-β-D-galactofuranosyl-(1→5)-2,3,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→4)-2,3-isopropylidene-α-L-rhamnopyranosyl-(1→3)-2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside (116). Prepared from **112** (48.0 mg, 0.08 mmol), donor **106** (139.5 mg, 0.12 mmol), activated, powdered 4 Å molecular sieves (250.0 mg), NIS (30.2 mg, 0.13 mmol), AgOTf (4.1 mg, 0.02 mmol) in CH_2Cl_2 (5 mL) as described for **70**. The resulting residue was purified by column chromatography (2:1 hexanes–EtOAc) to give tetrasaccharide **116** (94.4 mg, 75%) as an oil. R_f 0.37 (1:1 hexanes–EtOAc); $[\alpha]_D$ –20.6 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 8.03–7.92 (m, 12 H, Ar), 7.84–7.80 (m, 4 H, Ar), 7.57–7.10 (m, 24 H, Ar), 6.03 (ddd, 1 H, *J* = 7.5, 7.5, 5.0 Hz, H-5'''), 5.82 (dd, 1 H, *J* = 5.5, 1.2 Hz, H-3''), 5.81 (s, 1 H, H-1'''), 5.75 (s, 1 H, H-1''), 5.72 (d, 1 H, *J* = 10.0 Hz, NH), 5.65 (d, 1 H, *J* = 1.2 Hz, H-2''), 5.64 (dd, 1 H, *J* = 5.0, 1.3 Hz, H-3'''), 5.56 (d, 1 H, *J* = 1.3 Hz, H-2'''), 5.54 (s, 1 H, PhC*H*), 5.11 (s, 1 H, H-1'), 5.03 (dd, 1 H, *J* = 5.0, 5.0 Hz, H-4'''), 4.78–4.64 (m, 6 H, H-1, H-5'', H-6_a'', H-6_b'', H-6_a''', H-6_b'''), 4.45–4.40 (m, 2 H, H-4'', H-2), 4.30–4.27 (m, 2 H, H-3', H-6_a), 4.10–4.06 (m, 1 H, H-2'), 4.00 (dd, 1 H, *J* = 10.0, 10.0 Hz, H-

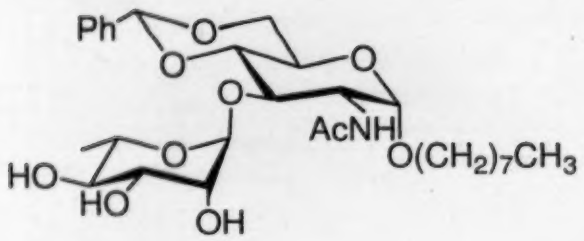
3), 3.96–3.92 (m, 1 H, H-5'), 3.86 (ddd, 1 H, J = 10.0, 10.0, 4.7 Hz, H-5), 3.78–3.69 (m, 2 H, H-6_b, octyl OCH_2), 3.61 (dd, 1 H, J = 10.0, 10.0 Hz, H-4), 3.57 (dd, 1 H, J = 10.0, 7.6 Hz, H-4'), 3.41 (ddd, 1 H, J = 9.8, 6.7, 6.7 Hz, octyl OCH_2), 2.03 (s, 3 H, C(=O)CH_3), 1.65–1.58 (m, 2 H, octyl CH_2), 1.53 (s, 3 H, isopropylidene CH_3), 1.39–1.25 (m, 13 H, octyl CH_2, isopropylidene CH_3), 0.90 (t, 3 H, J = 7.0 Hz, octyl CH_3), 0.86 (d, 3 H, J = 6.0 Hz, H-6'); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 169.8 (C=O), 166.1 (C=O), 165.9 (C=O), 165.7 (C=O), 165.6 (C=O), 165.5 (C=O), 165.3 (C=O), 165.2 (C=O), 133.4 (Ar), 133.3 (Ar), 133.2 (Ar), 133.1 (Ar), 133.0 (Ar), 132.9 (Ar), 132.8 (Ar), 130.0 (Ar), 129.9 (Ar), 129.81 (Ar), 129.76 (Ar), 129.73 (Ar), 129.7 (Ar), 129.63 (Ar), 129.6 (Ar), 129.0 (Ar), 128.97 (Ar), 128.9 (Ar), 128.8 (Ar), 128.7 (Ar), 128.5 (Ar), 128.4 (Ar), 128.3 (Ar), 128.28 (Ar), 128.2 (Ar), 128.1 (Ar), 128.0 (Ar), 127.9 (Ar), 109.3 ($(CH_3)_2C$), 105.5 (C-1'''), 104.0 (C-1''), 101.7 (PhCH), 98.2 (C-1), 98.1 (C-1'), 82.8 (C-4''), 82.2 (C-4'''), 82.0 (C-2'''), 81.9 (C-2''), 80.0 (C-4), 77.8 (C-3), 77.7 (C-3'''), 77.6 (C-3'), 76.4 (C-2'), 76.2 (C-4'), 74.8 (C-3''), 72.8 (C-5''), 70.5 (C-5'''), 68.9 (C-6), 68.3 (octyl OCH_2), 64.8 (C-6'''), 64.4 (C-5), 63.7 (C-6''), 63.1 (C-5'), 53.6 (C-2), 31.8 (octyl CH_2), 29.4 (octyl CH_2), 29.3 (octyl CH_2), 29.2 (octyl CH_2), 27.9 (C(=O)CH_3), 26.3 (isopropylidene CH_3), 26.2 (octyl CH_2), 23.4 (isopropylidene CH_3), 22.6 (octyl CH_2), 17.2 (C-6'), 14.1 (octyl CH_3). ESI-MS m/z calcd. for (M + Na) $C_{93}H_{97}NO_{27}Na$: 1682.6140. Found: 1682.6145.



117

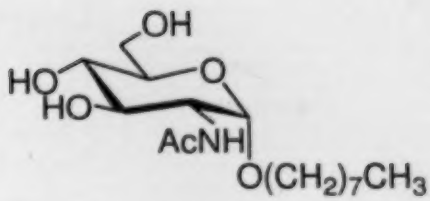
Octyl 2,3,4,5-tetra-*O*-benzoyl-β-D-galactofuranosyl-(1→6)-2,3,5-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→5)-2,3,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→4)-2,3-isopropylidene-α-L-rhamnopyranosyl-(1→3)-2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside (117). Prepared from **112** (12.3 mg, 0.02 mmol), donor **99** (50.1 mg, 0.03 mmol), activated, powdered 4 Å molecular sieves (150.0 mg), NIS (11.4 mg, 0.05 mmol) and AgOTf (2.6 mg, 0.01 mmol) in CH_2Cl_2 (5 mL) as described for **70**. The residue was purified by column chromatography (2:1 hexanes–EtOAc) to give pentasaccharide **117** (33.0 mg, 77%) as an oil. R_f 0.23 (1:1 hexanes–EtOAc); $[\alpha]_D$ –7.8 (*c* 0.2, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ_H) 8.01–7.92 (m, 13 H, Ar), 7.80 (dd, 4 H, *J* = 10.0, 3.0 Hz, Ar), 7.74 (d, 2 H, *J* = 7.5 Hz, Ar), 7.50–7.06 (m, 36 H, Ar), 6.02 (ddd, 1 H, *J* = 8.0, 8.0, 4.5 Hz, H-5'''), 5.91 (ddd, 1 H, *J* = 8.0, 8.0, 4.0 Hz, H-5''''), 5.81 (d, 1 H, *J* = 5.5 Hz, H-3''), 5.77 (s, 1 H, H-1''''), 5.75 (s, 1 H, H-1'''), 5.64–5.63 (m, 2 H, H-3'''', H-2''''), 5.56–5.53 (m, 3 H, H-3''', H-2'', H-1''), 5.32 (s, 1 H, H-2'''), 5.23 (s, 1 H, PhC*H*), 5.10 (s, 1 H, H-1'), 4.85 (dd, 1 H, *J* = 4.0 Hz, H-4''''), 4.73–4.65 (m, 8 H, H-

1, H-5'', H-4''', H-6_a'', H-6_b'', H-6_a''', H-6_b'''), 4.42–4.38 (m, 2 H, H-2, H-4''), 4.29–4.26 (m, 2 H, H-3', H-6_a), 4.12–4.08 (m, 2 H, H-2', H-6_a''''), 4.01–3.83 (m, 4 H, H-3, H-5', H-6_b'''', H-5), 3.77–3.68 (m, 2 H, H-6_b, octyl OCH_2), 3.61 (dd, 1 H, J = 9.0, 9.0 Hz, H-4), 3.54 (dd, 1 H, J = 10.0, 7.5 Hz, H-4'), 3.39 (ddd, 1 H, J = 9.5, 6.8, 6.8 Hz, octyl OCH_2), 2.02 (s, 3 H, C(=O)CH_3), 1.63–1.56 (m, 2 H, octyl CH_2), 1.43 (s, 3 H, isopropylidene CH_3), 1.33–1.26 (m, 13 H, octyl CH_2, isopropylidene CH_3), 0.92 (t, 3 H, J = 7.0 Hz, octyl CH_3), 0.83 (d, 3 H, J = 6.0 Hz, C-6'); ^{13}C NMR (125 MHz, $CDCl_3$, δ_C) 169.8 (C=O), 166.1 (C=O), 166.0 (C=O), 165.8 (C=O), 165.7 (C=O), 165.6 (C=O), 165.5 (C=O), 165.4 (C=O), 165.3 (C=O), 165.2 (C=O), 165.0 (C=O), 137.1 (Ar), 133.2 (Ar), 133.1 (Ar), 133.0 (Ar), 132.9 (Ar), 132.89 (Ar), 132.86 (Ar), 129.94 (Ar), 129.9 (Ar), 129.82 (Ar), 129.8 (Ar), 129.7 (Ar), 129.6 (Ar), 129.6 (Ar), 129.1 (Ar), 128.9 (Ar), 128.54 (Ar), 128.5 (Ar), 128.4 (Ar), 128.4 (Ar), 128.3 (Ar), 128.2 (Ar), 128.0 (Ar), 126.2 (Ar), 109.4 ($(CH_3)_2C$), 106.7 (C-1''''), 105.8 (C-1'''), 104.0 (C-1''), 101.7 (PhCH), 98.2 (C-1'), 98.1 (C-1), 82.6 (C-4''''), 82.0 (C-4'''), 81.9 (2 C, C-2''', C-2''), 81.8 (C-2''''), 80.1 (C-4), 77.8 (C-3''), 77.8 (C-3'), 77.6 (C-3''''), 77.4 (C-3'''), 76.4 (C-3), 76.2 (C-4'), 74.9 (C-2'), 73.1 (C-5''), 71.8 (C-5'''), 70.5 (C-5''''), 68.9 (C-6), 68.3 (octyl OCH_2), 67.7 (C-6''''), 64.9 (C-6'''), 64.4 (C-5'), 63.7 (C-6''), 63.2 (C-5), 53.6 (C-2), 31.8 (octyl CH_2), 29.7 (octyl CH_2), 29.4 (octyl CH_2), 29.3 (octyl CH_2), 29.2 (octyl CH_2), 27.9 (isopropylidene CH_3), 26.4 (isopropylidene CH_3), 26.2 (octyl CH_2), 23.4 (C(=O)CH_3), 22.6 (octyl CH_2), 17.2 (C-6'), 14.1 (octyl CH_3). ESI-MS m/z calcd. for (M + Na) $C_{120}H_{119}NO_{35}Na$: 2156.7460. Found: 2157.80.



118

Octyl α-L-rhamnopyranosyl-(1→3)-2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside (118). Prepared from **114** (88.5 mg, 0.13 mmol) in 3:1 MeOH–CH_2Cl_2 (5 mL) and NaOMe in MeOH (0.1 M) as described for **57**. The residue was purified by column chromatography (9:1 CH_2Cl_2–MeOH) to afford **118** (52.0 mg, 72%) as a semi-solid. R_f 0.24 (10:1 CH_2Cl_2–MeOH); $[\alpha]_D$ +3.9 (*c* 0.3, MeOH); ^{1}H NMR (500 MHz, CD_3OD, δ_H) 7.52–7.50 (m, 2 H, Ar), 7.39–7.34 (m, 3 H, Ar), 5.58 (s, 1 H, PhC*H*), 4.81 (br s, 1 H, H-1'), 4.71 (d, 1 H, J = 3.7 Hz, H-1), 4.22–4.18 (m, 2 H, H-2, H-4), 3.90 (dd, 1 H, J = 9.4, 9.4 Hz, H-3), 3.86–3.70 (m, 3 H, H-5, H-5', octyl O*CH*$_2$), 3.67 (dd, 1 H, J = 3.4, 1.8 Hz, H-2'), 3.65–3.61 (m, 2 H, H-3', H-4'), 3.57 (dd, 1 H, J = 9.6, 3.4 Hz, H-6$_a$), 3.45 (ddd, 1 H, J = 9.8, 6.4, 6.4 Hz, octyl O*CH*$_2$), 3.23 (dd, 1 H, J = 9.6, 9.6 Hz, H-6$_b$), 2.00 (s, 3 H, C(=O)*CH*$_3$), 1.69–1.64 (m, 2 H, octyl C*H*$_2$), 1.43–1.28 (m, 10 H, octyl C*H*$_2$), 0.90 (t, 3 H, J = 6.8 Hz, octyl C*H*$_3$), 0.70 (d, 3 H, J = 6.4 Hz, H-6'); ^{13}C NMR (125 MHz, CD_3OD, δ_C) 173.4 (C=O), 139.1 (Ar), 130.0 (Ar), 129.1 (Ar), 127.7 (Ar), 103.3 (PhCH), 102.6 (C-1), 99.6 (C-1'), 81.9 (C-4), 76.7 (C-3), 73.8 (C-4'), 72.5 (C-2'), 72.2 (C-6), 69.9 (C-3'), 69.8 (octyl O*CH*$_2$), 69.4 (C-5'), 64.5 (C-5), 55.3 (C-2), 33.0 (octyl *CH*$_2$), 30.52 (octyl *CH*$_2$), 30.51 (octyl *CH*$_2$), 30.4 (octyl *CH*$_2$), 27.3 (octyl *CH*$_2$), 23.7 (octyl *CH*$_2$), 22.6 (C(=O)*CH*$_3$), 17.6 (C-6'), 14.4 (octyl *CH*$_3$). ESI-MS *m/z* calcd for (M + Na) $C_{29}H_{45}NO_{10}Na$: 590.2936. Found. 590.2933.



119

Octyl 2-Acetamido-2-deoxy-α-D-glucopyranoside (119). To a suspension of *N*-acetyl-D-glucosamine **118** (4.42 g, 20 mmol) and *n*-octanol (9.45 mL, 60 mmol) in CH_3CN (160 mL), was added $BF_3{\cdot}OEt_2$ (500 μL, 3.5 mmol). The reaction mixture was stirred at reflux for 18 h. After cooling, the unreacted starting material was filtered, and the filtrate was evaporated to dryness. The residue was purified by column chromatography (10:1 CH_2Cl_2–MeOH) to give **119** as a solid (2.34 g, 35%). R_f 0.54 (10:1 CH_2Cl_2–MeOH). ^{1}H NMR (600 MHz, CD_3OD, δ_H) 4.78 (s, 1 H, J = 3.6 Hz, H-1), 3.88–3.85 (m, 1 H, octyl OCH_2), 3.80 (dd, 1 H, J = 10.6, 3.6 Hz, H-2), 3.71–3.64 (m, 3 H, H-3, H-4, H-5), 3.59–3.56 (m, 1 H, octyl OCH_2), 3.40–3.33 (m, 1 H, H-6_a, H-6_a), 1.97 (s, 3 H, NHCOCH_3), 1.60–3.58 (m, 2 H, octyl CH_2), 1.39–1.28 (m, 10 H, octyl CH_2), 0.89 (t, 3 H, J = 6.9 Hz, octyl CH_3), ESI-MS *m/z* calcd. for (M + Na) $C_{16}H_{31}NO_6Na$: 356.1071. Found: 356.1071.

BIBLIOGRAPHY

(1) Zink, A. R.; Sola, C.; Reischl, U.; Grabner, W.; Rastogi, N.; Wolf, H.; Nerlich, A. G. *Journal of Clin. Microbiol.* **2003**, *41*, 359–367.

(2) Salo, W. L.; Aufderheide, A. C.; Buikstra, J.; Holcomb, T. A. *Proc. Nat. Acad. Sci. U.S.A.* **1994**, *91*, 2091–2094.

(3) Kappelman, J.; Alcicek, M. C.; Kazanc, N.; Schultz, M.; Ozkul, M.; Sen, S. *Am. J. Phys. Anthropol.* **2008**, *135*, 110–116.

(4) Kaufmann, S. H.; Winau, F. *Nat. Immunol.* **2005**, *6*, 1063–1066.

(5) World Health Organization. *Global tuberculosis control: surveillance, planning, financing*; WHO: Geneva, **2006**.

(6) Corbett, E. L.; Watt, C. J.; Walker, N.; Maher, D.; Williams, B. G.; Raviglione, M. C.; Dye, C. *Arch. Intern. Med.* **2003**, *163*, 1009–1021.

(7) Gomez, J. E.; McKinney, J. D. *Tuberculosis (Edinb)* **2004**, *84*, 29–44.

(8) World Health Organization. *Global TB Control Report,*; WHO: Geneva, **2003**.

(9) *The Global MDR-TB and XDR-TB Response Plan 2007–2008*; World Health Organization and Stop TB Partnership, **2007**; also available at: http://whqlibdoc.who.int/hq/2007/WHO_HTM_TB_2007.387_eng.pdf. Last checked March 31, 2008.

(10) Medical News Today. *Extensively Drug-Resistant Tuberculosis (Xdr-Tb): The Facts*, **2007**; also available at: http://www.medicalnewstoday.com/articles/66187.php. Last checked March 31, 2008.

(11) Schwartz, J. In *Tangle of Conflicting Accounts In TB Patient's 12-Day Odyssey, New York Times,* June 2, 2007, p A1.

(12) Altman, K. In *Traveler's TB Not as Severe as Officials Thought , New York Times,* July 4, 2007, p A11.

(13) Nathanson, E.; Lambregts-van Weezenbeek, C.; Rich, M. L.; Gupta, R.; Bayona, J.; Blöndal, K.; Caminero, J. A.; Cegielski, J. P.; Danilovits, M.; Espinal, M. A.; Hollo, V.; Jaramillo, E.; Leimane, V.; Mitnick, C. D.; Mukherjee, J. S.; Nunn, P.; Pasechnikov, A.; Tupasi, T.; Wells, C.; Raviglione, M. C. *Emerg. Infect. Dis.* **2006**, *12*, 1389–1397.

(14) *Drug-Resistant TB*; Global Alliance for TB Drug Development, **2007**, also available at: http://www.tballiance.org/why/mdr-tb.php. Last checked March 31, 2008

(15) World Health Organization. *Emergence of XDR-TB*; WHO: Geneva, **2006**, also available at: http://www.who.int/mediacentre/news/notes/2006/np23/en/index.html. Last checked March 31, 2008.

(16) Gandhi, N. R.; Moll, A.; Sturm, A. W.; Pawinski, R.; Govender, T.; Lalloo, U.; Zeller, K.; J.;, A.; Friedland, G. *Lancet* **2006**, *368*, 1575–1580.

(17) World Health Organization. *The World Health Organization Global Tuberculosis Program*; WHO: Geneva, **2003**.

(18) Wade, M. M.; Zhang, Y. *Front. Biosci.* **2004**, *9*, 975–994.

(19) Blanchard, J. S. *Annu. Rev. Biochem.* **1996**, *65*, 215–239.

(20) Winder, F. G.; Collins, P. B. *J. Gen. Microbiol.* **1970**, *63*, 41–48.

(21) Telenti, A.; Philipp, W.; Sreevatsan, S.; Bernasconi, C.; Stockbauer, K. E.; Wieles, B.; Musser, J.; Jacobs Jr., W. *Nat. Med.* **1997**, *3*, 567–570.

(22) Zhang, Y. *Annu. Rev. Pharmacol. Toxicol.* **2005**, *45*, 529–564.

(23) Garvin, R. T.; Biswas, D. K.; Gorini, L. *Proc. Natl. Acad. Sci. U.S.A.* **1974**, *71*, 3814–3818

(24) Zhang, Y.; Wade, M. M.; Scorpio, A.; Zhang, H.; Sun, Z. *J. Antimicrob. Chemother.* **2003**, *52*, 790–95.

(25) Zimhony, O.; Vilche`ze, C.; Arai, M.; Welch, J. T.; Jacobs, J. W. R. *Antimicrob. Agents Chemother.* **2007**, *51*, 752–754.

(26) Russell, D. G. *Nat. Rev. Immunol.* **2007**, *5*, 39–47.

(27) Zahrt, T. C. *Microbes Infect.* **2003**, *5*, 159–167.

(28) Mitchison, D. A. *Chest* **1979**, *76*, 771–781.

(29) Jindani, A.; Dore, C. J.; Mitchison, D. A. *Am. J. Resp. Crit. Care* **2003**, *167*, 1348–1354.

(30) Brennan, P. J.; Nikaido, H. *Annu. Rev. Biochem.* **1995**, *64*, 29–63.

(31) Pan, F.; Jackson, M.; Ma, Y.; McNeil, M. J. *Bacteriol. Rev.* **2001**, *183*, 3991–3998.

(32) Mills, J. A.; Motichka, K.; Jucker, M.; Wu, H. P.; Uhlik, B. C.; Stern, R. J.; Scherman, M. S.; Vissa, V. D.; Pan, F.; Kundu, M.; Ma, Y. F.; McNeil, M. *J. Biol. Chem.* **2004**, *279*, 43540–43546.

(33) Vilcheze, C.; Morbidoni, H. R.; Weisbrod, T. R.; Iwamoto, H.; Kuo, M.; Sacchettini, J. C.; Jacobs, W. R., Jr. *J. Bacteriol.* **2000**, *182*, 4059–4067.

(34) Bhamidi, S.; Scherman, M. S.; Rithner, C. D.; Prenni, J. E.; Chatterjee, D.; Khoo, K. H.; McNeil, M. R. *J. Biol. Chem.* **2008**, *In press;* also available at: http://www.jbc.org/cgi/doi/10.1074/jbc.M800222200.

(35) Takayama, K.; Kilburn, J. D. *Antimicrob. Agents Chemother.* **1989**, *33*, 1493–1499.

(36) Singh, S.; Hogan, S. E. *Microbios* **1994**, *77*, 217–222.

(37) Crick, D. C.; Brennan, D. J. *Curr. Opin. Anti-Infect. Inves. Drugs* **2000**, *2*, 537–544.

(38) Berg, S.; Kaur, D.; Jackson, M.; Brennan, P. J. *Glycobiology* **2007**, *17*, 35R–56R.

(39) Jarlier, V.; Nikaido, H. *J. Bacteriol.* **1990**, *172*, 1418–1423.

(40) Trias, J.; Benz, R. *Mol. Microbiol.* **1994**, *14*, 283–290.

(41) Chambers, H. F.; Moreau, D.; Yajko, D.; Miick, C.; Wagner, C.; Hackbarth, C.; Kocagoz, S.; Rosenberg, E.; Hadley, W. K.; Nikaido, H. *Antimicrob. Agents Chemother.* **1995**, *39*, 2620–2624.

(42) Jarlier, V.; Gutmann, L.; Nikaido, H. I. *Antimicrob. Agents Chemother.* **1991**, *35*, 1937–1939

(43) Barry, C. E., III; ; Mdluli, K. *Trends Microbiol.* **1996**, *4*, 275–281.

(44) Brennan, P. J. In *Microbial Lipids*; Ratledge, C., Wilkinson, S. G., Eds.; Academic Press Limited: London, 1988, p 203–298.

(45) Crick, D. C.; Mahapatra, S.; Brenann, P. J. *Glycobiology* **2001**, *11*, 107R–118R.

(46) Brennan, P. J. *Tuberculosis (Edinb)* **2003**, *83*, 91–97.

(47) Lowary, T. L. In *Glycoscience: Chemistry and Chemical Biology*; Springer-Verlag: Berlin, 2001, p 2005–2080.

(48) Brennan, P. J. *Rev. Infect. Dis.* **1989**, *11*, 420–430.

(49) Minnikin, D. In *The Biology of Mycobacteria*; Ratledge, C., Stanford, J., Eds.; Academic Press: London, 1982; Vol. 1, p 95.

(50) Lederer, E.; Adam, A.; Ciorbaru, R.; Petit, F.; Wietzerbin, J. *Mol. Cell. Biochem.* **1975**, *7*, 87–104.

(51) Ghuysen, J. M. *Bacteriol. Rev.* **1968**, *32*, 425–464.

(52) McNeil, M.; Wallner, S. J.; Hunter, S. W.; Brennan, P. J. *Carbohydr Res.* **1987**, *166*, 299–308.

(53) Daffe, M.; McNeil, m.; Brennan, P. J. *Carbohydr. Res.* **1993**, *249*, 383–398.

(54) Alderwick, L. J.; Radmacher, E.; Seidel, M.; Gande, R.; Hitchen, P. G.; Morris, H. R.; Dell, A.; Sahm, H.; Eggeling, L.; Besra, G. S. *J. Biol. Chem.* **2005**, *280*, 32362–32371.

(55) Lee, A.; Wu, S. W.; Scherman, M. S.; Torrelles, J. B.; Chatterjee, D.; McNeil, M. R.; Khoo, K. H. *Biochemistry* **2006**, *45*, 15817–15828.

(56) McNeil, M.; Daffe, M.; Brennan, P. J. *J. Biol. Chem.* **1991**, *266*, 13217–13223.

(57) Brennan, P. J.; Besra, G. S.; 1997, 188 *Biochem. Soc. Trans.* **1997**, *25*, 188–194.

(58) Liu, J.; Barry, C. E.; Besra, G. S.; Nikaido, H.; *J. Biol. Chem.* **1996**, *271*, 29545–29551.

(59) Barry, C. E.; Lee, R. E.; Mdluli, K.; Sampson, A. E.; Schroeder, B. G.; Slayden, R. A.; Yuan, Y. *Prog. Lipid Res.* **1998**, *37*, 143–179.

(60) Yuan, Y.; Crane, D. C.; Musser, J. M.; Sreevatsan, S.; Barry, C. E. *J. Biol. Chem.* **1997**, *272*, 10041–10049.

(61) Fontecave, M.; Eliasson, R.; Reichard, P. M. *J. Biol. Chem.* **1987**, *262*, 12325–12331.

(62) Nigou, J.; Vercellone, A.; Puzo, G. *J. Mol. Biol.* **2000**, *299*, 1353–1362.

(63) Joe, M.; Sun, D.; Taha, H.; Completo, G. C.; Croudace, J. E.; Lammas, D. A.; Besra, G. S.; Lowary, T. L. *J. Am. Chem. Soc.* **2006**, *128*, 5059–5072

(64) Chatterjee, D.; Khoo, K.-H. *Glycobiology* **1998**, *8*, 113–120.

(65) Khoo, K.-H.; Dell, A.; Morris, H. R.; Brennan, P. J.; Chatterjee, D. *J. Biol. Chem.* **1995**, *270*, 12380–12389.

(66) Prinzis, S.; Chatterjee, D.; Brennan, P. J. *J. Gen. Microbiol.* **1993**, *139*, 2649–2658.

(67) Besra, G. S.; Chatterjee, D. In *Tuberculosis: Pathogenesis, Protection, and Control*; Bloom, B. R., Ed.; ASM Press: Washington, D.C., 1994, p 285–306.

(68) Aspinall, G. O.; Chatterjee, D.; Brennan, P. J. *Adv. Carbohydr. Chem. Biochem.* **1995**, *51*, 169–242.

(69) Yagi, T.; Mahapatra, S.; Mikušová, K.; Crick, D. C.; Brennan, P. J. *J. Biol. Chem.* **2003**, *278*, 26497–26504.

(70) Berg, S.; Starbuck, H.; Torrelles, J. B.; Vissa, V. D.; Crick, D. C.; Chatterjee, D.; Brennan, P. J. *J. Biol. Chem.* **2005**, *280*, 5651–5663.

(71) Alderwick, L. J.; Seidel, M.; Sahm, H.; Besra, G. S.; Eggeling, L. *J. Biol. Chem.* **2006**, *281*, 15653–15661.

(72) Belanger, A. E.; Besra, G. S.; Ford, M. E.; Mikušová, K.; Belisle, J. T.; Brennan, P. J.; Inamine, J. M. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 11919–11924.

(73) Hancock, I. C.; Carman, S.; Besra, G. S.; Brennan, P. J.; Waite, E. *Microbiology* **2002**, *148*, 3059–3067.

(74) Kremer, L.; Dover, L. G.; Morehouse, C.; Hitchin, P.; Everett, M.; Morris, H. R.; Dell, A.; Brennan, P. J.; McNeil, M. R.; Flaherty, C.; Duncan, K.; Besra, G. S. *J. Biol. Chem.* **2001**, *27*, 26430–26440.

(75) Mikušová, K.; Yagi, T.; Stern, R.; McNeil, M. R.; Besra, G. S.; Crick, D. C.; Brennan, P. J. *J. Biol. Chem.* **2000**, *275*, 33890–33897.

(76) Rose, N. L.; Completo, G. C.; Lin, S.-J.; McNeil, M. R.; Palcic, M. M.; Lowary, T. L. *J. Am. Chem. Soc.* **2006**, *128*, 6721–6729.

(77) Seidel, M.; Alderwick, L. J.; Birch, H. L.; Sahm, H.; Eggeling, L.; Besra, G. S. *J. Biol. Chem.* **2007**, *282*, 14729–14740.

(78) Crick, D. C.; Schulbach, M. C.; Zink, E. E.; Macchia, M.; Barontini, S.; Besra, G. S.; Brennan, P. J. *J. Bacteriol.* **2000**, *182*, 5771–5778.

(79) McNeil, M. *Arabinogalactan in mycobacteria: structure, biosynthesis, and genetics*; CRS Press: Washington, D. C., 1999.

(80) Mikušová, , K.; Mikus, M.; Besra, G. S.; Hancock, I.; Brennan, P. J. *J. Biol. Chem.* **1996**, *271*, 7820–7828.

(81) Weston, A.; Stern, R. J.; R. E. Lee, R. E.; Nassau, P. M.; Monsey, D.; Martin, S. L.; Scherman, M. S.; Besra, G. S.; Duncan, K.; McNeil, M. R. *Tuber. Lung Dis.* **1997**, *78*, 123–131.

(82) Mikušová, K.; Beláňová, M.; Kordulakova, J.; Honda, K.; McNeil, M. R.; Mahapatra, S.; Crick, D. C.; Brennan, P. J. *J. Bacteriol.* **2006**, *188*, 6592–6598.

(83) Nassau, P. M.; Martin, S. L.; Brown, R. E.; Weston, A.; Monsey, D.; McNeil, M.; Duncan, K. *J. Bacteriol.* **1996**, *178*, 1047–1052.

(84) Beláňová, M.; Dianiskova, P.; Brennan, P. J.; Completo, G. C.; Rose, N. T.; Lowary, T. L.; Mikušová, K. *J. Bacteriol.* **2008**, *190*, 1141–1145.

(85) Wolucka, B. A.; McNeil, M. R.; de Hoffmann, E.; Chojnacki, T.; Brennan, P. J. *J. Biol. Chem.* **1994**, *269*, 23328–23335.

(86) Xin, Y.; Lee, R. E.; Scherman, M. S.; Khoo, K. H.; Besra, G. S.; Brennan, P. J.; McNeil, M. *Biochim. Biophys. Acta* **1997**, *1335*, 231–234.

(87) Lee, R. E.; Brennan, P. J.; Besra, G. S. *Glycobiology* **1997**, *7*, 1121–1128.

(88) Lee, R. E.; Mikušová, K.; Brennan, P. J.; Besra, G. S. *J. Am. Chem. Soc.* **1995**, *117*, 11829–11832.

(89) Bhatt, A.; Kremer, L.; Dai, A. Z.; Sacchettini, J. C.; Jacobs, W. R., Jr. *J. Bacteriol.* **2005**, *187*, 7596–7606.

(90) Portevin, D.; de Sousa-D'Auria, C.; Montrozier, H.; Houssin, C.; Stella, A.; Laneelle, M. A.; Bardou, F.; Guilhot, C.; Daffe, M. *J. Biol. Chem.* **2005**, *280*, 8862–8874.

(91) Breton, C.; Snajdrová, L.; Jeanneau, C.; Koca, J.; Imberty, A. *Glycobiology* **2006**, 16, 29R–37R.

(92) Chiu, C. P. C.; Watts, A. G.; Lairson, L. L.; Gilbert, M.; Lim, D.; Wakarchuk, W. W.; Withers, S. G.; Strynadka, N. C. J. *Nat. Struct. Mol. Biol.* **2004**, *11*, 163–170.

(93) Tsvetkov, Y. E.; Nikolaev, A. V. *J. Chem. Soc. Perkin Trans. 1* **2000**, 889–891.

(94) Zhang, Q.; Liu, H.-W. *J. Am. Chem. Soc.* **2000**, *122*, 9065–9070.

(95) Zhang, Q.; Liu, H.-W. *J. Am. Chem. Soc.* **2001**, *123*, 6756–6766.

(96) Zhang, Q.; Liu, H.-W. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 145–149.

(97) Marlow, A. L.; Kiessling, L. L. *Org. Lett.* **2001**, *3*, 2517–2519.

(98) Rose, N. L.; Pearcey, J. A.; Zheng, R. B.; Zhou, R.; Completo, G. C.; Lowary, T. L. *Carbohydr. Res.* **2008**, *accepted.*

(99) Lubineau, A.; Fischer, J.-C. *Synth. Commun.* **1991**, *21*, 815–818.

(100) Arasappan, A.; Fraser-Reid, B. *Tetrahedron Lett.* **1995**, *36*, 7967–7070.

(101) Velty, R.; Benvegnu, T.; Gelin, M.; Privat, E.; Plusquellec, D. *Carbohydr. Res.* **1997**, *299*, 7–14.

(102) D'Accorso, N. B.; Thiel, I. M. E.; Schuller, M. *Carbohydr. Res.* **1983**, *124*, 177–184.

(103) Green, J. W.; Pacsu, E. J. *J. Am. Chem. Soc.* **1938**, *87*, 5469–5472.

(104) McAuliffe, J. C.; Hindsgaul, O. *J. Org. Chem.* **1997**, *62*, 1234–1239.

(105) Kovensky, J.; Sinay, P. *Eur. J. Org. Chem.* **2000**, 3523–2525.

(106) Morgenlie, S. *Acta Chem. Scand.* **1973**, *27*, 3609–3611.

(107) Wang, H.; Zhang, G.; Ning, J. *Carbohydr. Res.* **2003**, *338*, 1033–1037.

(108) Rauter, A. P.; Ramoa-Riberio, F.; Fernanders, A. C.; Figueiredo, J. A. *Tetrahedron* **1995**, *51*, 6529–6532.

(109) Capon, B. *Chem. Rev.* **1969**, *69*, 407–498.

(110) Angyal, S. J. *Chem. Soc. Rev.* **1980**, *9*, 415–428.

(111) Biemann, K.; DeJongh, D. C.; Schnoes, H. K. *J. Am. Chem. Soc.* **1963**, *85*, 1763–1771.

(112) Jarosz, S.; Krajewski, J. W.; Zamojski, A.; Duddeck, H.; Kaiser, M. *Bull. Pol. Acad. Sci, Chem.* **1985**, *33*, 181–187.

(113) De Jongh, D. C.; Biemann, K. *J. Am. Chem. Soc.* **1964**, *86*, 67–74.

(114) Fischer, E. *Ber.* **1894**, *27*, 673–679.

(115) Green, J. W. *Adv. Carbohydr. Chem. Biochem.* **1966**, *21*, 95–142.

(116) Pascu, E.; Green, J. W. *J. Am. Chem. Soc.* **1936**, *58*, 1823–1824.

(117) Wolfrom, M. L.; Weisblat, D. I.; Hanze, A. R. *J. Am. Chem. Soc.* **1944**, *66*, 2065–2068.

(118) Zemplén, G.; Mester, L.; Messmer, A. *Chem. Ber.* **1953**, *86*, 697–699.

(119) Madhusudan, S. K.; Misra, A. K. *Carbohydr. Res.* **2005**, *340*, 497–502.

(120) Szarek, W. A.; Zamojski, A.; Tiwari, K. N.; Ison, E. R. *Tetrahedron Lett.* **1986**, *27*, 3827–3830.

(121) Kartha, R. P. R.; Aloui, M.; Field, R. A. *Tetrahedron Lett.* **1996**, *37*, 5175–5178.

(122) Vaino, A. R.; Szarek, W. A. *J. Chem. Soc. Chem. Commun.* **1996**, 2351–2352.

(123) Wahlstrom, J. L.; Ronald, R. C. *J. Org. Chem.* **1998**, *63*, 6021–6022.

(124) Ramasamy, K. S.; Bandaru, R.; Averett, D. *Synth. Commun.* **1999**, *29*, 2881–2894.

(125) Palcic, M. M.; Heerze, L. D.; Pierce, M.; Hindsgaul, O. *Glycoconjugate J.* **1988**, *5*, 49–63.

(126) Garegg, P. J. *Adv. Carbohydr. Chem. Biochem.* **2004**, *59*, 69–134.

(127) Kihlberg, J. O.; Leigh, D. A.; Bundle, D. R. *J. Org. Chem.* **1990**, *55*, 2860–2863.

(128) Lonn, H. *J. Carbohydr. Chem.* **1987**, *6*, 301–306.

(129) Andersson, F.; Fugedi, P.; Garegg, P. J.; Nashed, M. *Tetrahedron Lett.* **1986**, *27*, 3919–3922.

(130) Veeneman, G. H.; van Boom, J. H. *Tetrahedron Lett.* **1990**, *31*, 275–278.

(131) Veeneman, G. H.; van Leeuwen, S. H.; van Boom, J. H. *Tetrahedron Lett.* **1990**, *31*, 1331–1334.

(132) Konradsson, P.; Udodong, U. E.; Fraser-Reid, B. *Tetrahedron Lett.* **1990**, *31*, 4313–4316.

(133) Crich, D.; Smith, M. *J. Am. Chem. Soc.* **2001**, *123*, 9015–9020.

(134) Codee, J. D.; Litjens, R. E. J. N.; den Heeten, R.; Overkleeft, H. S.; van Boom, J. H.; van der Marel, G. A. *Org. Lett.* **2003**, *5*, 1519–1522.

(135) Kohn, P.; Samaritano, R. H.; Lerner, L. M. *J. Am. Chem. Soc.* **1965**, *87*, 5475–5480.

(136) Wolfrom, M. L. *J. Am. Chem. Soc.* **1930**, *52*, 2464–2473.

(137) Cyr, N.; Perlin, A. S. *Can. J. Chem.* **1979**, *57*, 2504–2511.

(138) Marino, C.; Marino, K.; Miletti, L.; Alves, M. J. M.; Colli, W.; Lederkremer, R. M. *Glycobiology* **1998**, *8*, 901–904.

(139) Zunszain, P. A.; Varela, O. *J. Chem. Res.* **1995**, (S) 486–487, (M) 2910–2913

(140) Callam, C. S.; Lowary, T. L. *J. Chem. Educ.* **2001**, *78*, 73–74.

(141) Mizutani, K.; Kasai, R.; Nakamura, M.; Tanaka, O.; Matsuura, H. *Carbohydr. Res.* **1989**, *185*, 27–38.

(142) Pathak, A. K.; Yahya, E. A.; Maddry, J. A.; Reynolds, R. C. *Tetrahedron Lett.* **1998**, *39*, 1497–1500.

(143) Pathak, A. K.; Pathak, V.; Seitz, L.; Maddry, J. A.; Gurcha, S. S.; Besra, G. S.; Suling, W. J.; Reynolds, R. C. *Bioorg. Med. Chem.* **2001**, *9*, 3129–3143.

(144) Ferrieres, V.; Plusquellec, D. J. *Carbohydr. Res.* **1998**, *311*, 25–35.

(145) Choudhury, A. K.; Roy, N. *Carbohydr. Res.* **1998**, *308*, 207–211.

(146) Byramova, N. E.; Ovchinnikov, M. V.; Bachinowsky, L. V.; Kochetkov, N. K. *Carbohydr. Res.* **1983**, *124*, C8–C11.

(147) Zhang, Z.; Ollman, I. R.; Ye, X. S.; Wischnat, R.; Baasov, T.; Wong, C.-H. *J. Am. Chem. Soc.* **1999**, *121*, 734–753.

(148) Mong, T. K.-K.; Lee, H.-K.; Duron, S. G.; Wong, C.-H. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 797–802.

(149) Burkhart, F.; Zhang, Z.; Wacowich-Sgarbi, S.; Wong, C.-H. *Angew. Chem.* **2001**, *113*, 1314–1317.

(150) Yamada, H.; Harada, T.; Takahashi, T. *J. Am. Chem. Soc.* **1994**, *116*, 7919–7920.

(151) Haines, A. H. *Adv. Carbohydr. Chem. Biochem.* **1976**, *33*, 11–109.

(152) Gallo-Rodriguez, C.; Gandolfi, L.; de Lederkremer, R. M. *Org. Lett.* **1999**, *1*, 245–247.

(153) Schmidt, R. R.; Kinzy, W. *Adv. Carbohydr. Chem. Biochem.* **1994**, *50*, 21–123.

(154) Fugedi, P.; Liptak, A.; Nanasi, P. *Carbohydr. Res.* **1982**, *107*, C5–C8.

(155) Isobe, M.; Takahashi, H.; Goto, T. *Tetrahedron Lett.* **1990** *31*, 717–718.

(156) Gallo-Rodriguez, C.; Gil-Libarona, M. A.; Mendoza, V. M.; de Lederkremer, R. M. *Tetrahedron* **2002**, *58*, 9373–9380.

(157) Gandolfi-Donadio, L.; Gallo-Rodriguez, C.; de Lederkremer, R. M. *J. Org. Chem.* **2002**, *67*, 4430–4435.

(158) Gandolfi-Donadio, L.; Gallo-Rodriguez, C.; de Lederkremer, R. M. *J. Org. Chem.* **2003**, *68*, 6928–6934.

(159) Zhang, G.; Fu, M.; Ning, J. *Tetrahedron Assym.* **2005**, *16*, 733–738.

(160) Zhang, G.; Fu, M.; Ning, J. *Carbohydr. Res.* **2005**, *340*, 155–159.

(161) Codee, J. D.; van den Bos, L. J.; Litjens, R. E. J. N.; Overkleeft, H. S.; van Boom, J. H.; van der Marel, G. A. *Org. Lett.* **2003**, *5*, 1947–1950.

(162) Wang, Y.; Zhang, L.-H.; Ye, X.-S. *Comb. Chem. High T. Scr.* **2006**, *9*, 63–75.

(163) Codée, J. D. C.; Litjens, R. E. J. N.; Van Den Bos, L. J.; Overkleeft, H. S.; Van Der Marel, G. A. *Chem. Soc. Rev.* **2005**, *34*, 769–782.

(164) Seeberger, P. H.; Werz, D. B. *Nature* **2007**, *446*, 1046–1051.

(165) Mong, T. K.-K.; Wong, C.-H. *Angew. Chem. Int. Ed.* **2002**, *41*, 4087–4090.

(166) Yamada, H.; Harada, T.; Miyasaki, H.; Takahashi, T.; *Tetrahedron Lett.* **1994**, *35*, 3979–3982.

(167) Yu, B.; Hui, H.; Han, X. *Tetrahedron Lett.* **1999**, *40*, 8591–8594.

(168) Wang, H.; Ning, J. *J. Org. Chem.* **2003**, *68*, 2521–2524.

(169) Sanders, D. A. R.; Staines, A. G.; McMahon, S. A.; McNeil, M. R.; Whitfield, C.; Naismith, J. H. *Nat. Struct. Biol.* **2001**, *8*, 858–863.

(170) Daffe, M.; Brennan, P. J.; McNeil, M. *J. Biol. Chem.* **1990**, *265*, 6734–6743.

(171) McNeil, M.; Daffe, M.; Brennan, P. J. *J. Biol. Chem.* **1990**, *265*, 18200–18206.

(172) Aguilera, B.; Romero-Ramirez, L.; Abad-Rodriguez, J.; Corrales, G.; Nieto-Sampedro, M.; Fernandez-Mayoralas, A. *J. Med. Chem.* **1998**, *41*, 4599–4606.

(173) Sawada, T.; Fujii, S.; Nakano, H.; Ohtake, S.; Kimata, K.; Habuchi, O. *Carbohydr. Res.* **2005**, *340*, 1983–1996.

(174) Bock, K.; Pedersen, C. *J. Chem. Soc., Perkin Trans.* **1974**, *2*, 293–297.

(175) Patani, G. A.; Lavoie, E. J. *Chem. Rev.* **1996**, *96*, 3147–3176.

(176) Rye, C. S.; Baell, J. B. *Curr. Med. Chem.* **2005**, *12*, 3127–3141.

(177) Charnock, S. J.; Davies, G. J. *Biochemistry* **1999**, *38*, 6380–6385.